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THE EFFECT OF CORTISONE ON THE LESIONS OF PERIARTERITIS NODOSA *

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When cortisone was found to be effective in controlling many of the symptoms and signs of rheumatoid arthritis [1] and acute rheumatic fever,[2] it was postulated that an ameliorating effect might also be expected in periarteritis nodosa. These three diseases, together with some others, have been grouped by some investigators [3-6] under the broad but somewhat inaccurate term "diffuse collagen diseases." Although it is well understood that these diseases differ from one another in many clinical and pathologic aspects, the opinion that a common pathogenesis exists has been frequently expressed. Consequently, the fact that cortisone had been effective in two of these conditions was a sufficient stimulus to investigate its effect on periarteritis nodosa. This report is concerned with 2 cases of histologically verified periarteritis nodosa in which cortisone was administered and which were studied at necropsy.

REPORT OF CASES

CASE 1

The patient was a physician, 45 years of age, who was admitted to the hospital on July 18, 1949, with an established diagnosis of periarteritis nodosa. He had undergone gastro-enterostomy in 1939 for an obstructing duodenal ulcer. In June, 1948, he had an injection of tetanus antitoxin, following which a severe urticarial reaction developed. This was the third or fourth injection of antitetanic serum he had received during his life, and apparently the second time he had reacted adversely to it. There was also a history of a vascular hyper-reactivity with transient elevations of blood pressure up to 180/100 mm. of Hg during the past 10 years.

The patient was hospitalized elsewhere from May 12 to 20, because of abdominal pain which gradually subsided with conservative treatment. Shortly after he had returned to his home, muscular aching and soreness and a few small, tender, subcutaneous nodules developed in his extremities. On June 12, he was again hospitalized elsewhere, where he remained until coming to the Clinic. Specimens taken for biopsy of one of the subcutaneous nodules and the right gastrocnemius muscle on June 15

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and 20 respectively showed lesions which were considered to be characteristic of periarteritis nodosa (Fig. 1).

On admission to the Mayo Clinic the patient appeared chronically ill. His blood pressure was 145/90 mm. of Hg. Other pertinent physical findings included marked generalized weakness with pronounced muscular tenderness and atrophy of the extremities, mild generalized lymphadenopathy and a systolic murmur of moderate intensity, audible over the apex. There were no subcutaneous nodules and no evidence of peripheral neuropathy.

The electrocardiogram showed minor alterations of the T waves in the standard limb leads consistent with myocardial damage. Blood studies revealed a mild anemia and hypoproteinemia, slight decreases in concentration of plasma chlorides, serum sodium and potassium, and a concentration of urea of 30 mg. per 100 cc. of blood. There was a 2 per cent retention of sulfobromophthalein sodium. The urine showed a specific gravity of 1.010, with albumin, grade 2. Biopsy of a painful region in the left gastrocnemius muscle confirmed the diagnosis of periarteritis nodosa (Fig. 2).

During his first 10 days in the hospital the patient had daily temperature elevations of 99° to 101.4° F. He was unable to sit up in bed without assistance and had to be fed. Leukocytes numbered 17,500 to 24,200 per cmm., and his sedimentation rates (Westergren method) varied between 101 and 117 mm. in 1 hour. Administration of cortisone acetate * was started on July 28, 10 days after his admission to the hospital, and was continued daily for 25 days. The total dosage in this period was 2.8 gm. Clinical improvement was prompt and dramatic and within 48 hours the patient was afebrile, virtually free of muscular pain and tenderness, and was able to feed himself and sit up without assistance. There was a gradual decrease of the sedimentation rate to 38 mm. in 1 hour on August 17, 3 weeks after the administration of cortisone was begun. However, leukocytosis persisted. Later, both the leukocyte count and sedimentation rate were within normal limits on a few occasions prior to the patient's death.

After 1 week of administration of cortisone (average daily dose, 142.8 mg.) symptoms and signs of congestive heart failure developed, necessitating digitalization and the occasional use of mercurial diuretics. Following what appeared to be restoration of cardiac compensation, the patient's general status improved. However, on August 22, after 25 days of administration of cortisone, the patient had a severe attack of pulmonary edema. The first course of cortisone was therefore discontinued after August 21. The pulmonary edema responded satisfactorily to the usual measures, but during the next 9 days he was in and out of congestive heart failure and on one occasion had a transient pericardial friction rub. It became necessary to maintain him on rather large doses of digitoxin and to resort to more frequent use of mercurial diuretics. There was no change in weight (120 lbs.) or in urinary output (2 to 3 l. daily) after stopping cortisone. However, he remained afebrile and free of muscular pains. On September 5, 15 days after discontinuance of cortisone, a few tender subcutaneous nodules appeared over the left side of the chest. Biopsy of one of these nodules again showed active lesions of periarteritis nodosa (Fig. 3). Further trial of cortisone was considered to involve the risk of additional cardiac embarrassment but, because of the evidences of recurrent activity of the primary disease, the use of cortisone in the dosage of 75 mg. daily was resumed on September 9.

The patient's subsequent course was progressively unfavorable with increasing azotemia and decreasing concentrations of plasma chlorides, serum sodium, and serum potassium, accompanied by increasing mental confusion. Use of cortisone

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was discontinued on September 19, after 11 days of administration during the second course. The total dosage in this period was 0.825 gm. Despite attempts to correct the plasma electrolyte deficit, the patient lapsed into coma, uremia developed, and he died on October 1.

Necropsy

At necropsy, the left pleural cavity contained 20 cc. and the right, 400 cc. of clear yellow fluid. The pericardial sac was almost completely obliterated by fine fibrinous adhesions. The heart weighed 520 gm. The myocardium was flabby, and all chambers were dilated. There was a gray mural thrombus, which measured 8 mm. in greatest extent, in the posterosuperior portion of the right auricle. The mitral ring was dilated and admitted 3 fingers. The mitral leaflets were vascularized, irregularly thickened, and scarred. Small thrombi were attached to the line of closure on irregularly thickened and roughened areas. The chordae tendineae were thickened but not shortened. The aortic ring was dilated also, and the three aortic cusps were thickened and adherent to one another. The cut surface of the myocardium of both ventricles revealed numerous small, white scars and irregular hemorrhagic areas.

In the apex of the upper lobe of the right lung there was a partially calcified, gray, nodular area 1 cm. in the greatest extent. Scattered throughout the upper lobe there were tiny, grayish, firm nodules, and in the right lower lobe another partially calcified, nodular area 0.8 cm. in diameter. The liver was somewhat atrophied and weighed 1,410 gm. In the right lobe of the liver near the hilus there was a dark hemorrhagic area 2 by 2 by 4 cm.

The gastrojejunal stoma was patent, with a diameter of 1 cm. On the jejunal surface 1 cm. from the anastomotic line there was a deep, active ulcer 0.5 cm. in diameter. In the duodenum there was a stellate scar 3 cm. distal to the pylorus and an active ulcer 0.7 cm. in diameter with thick, indurated edges was found 2 cm. distal to the pylorus. In the midportion of the jejunum there were two ulcers, each 0.6 cm. in diameter, and in the upper portion of the ileum, one ulcer 1 cm. in diameter. In the colon at the splenic flexure three ulcers, each approximately 0.6 cm. in diameter, were observed. All of the intestinal ulcers had gray, indurated bases.

The adrenal glands appeared smaller than normal. The right adrenal weighed 6.5 gm. and the left 6 gm. The cortices appeared narrowed.

The external surfaces of both kidneys were made irregular by alternating depressed and elevated areas. The cut surface revealed

some of the depressed areas to be congested and hemorrhagic, whereas others appeared scarred and bloodless. The cortex measured 0.6 cm. and the medulla 1.5 cm. in thickness. In the midportion of the left kidney there was a red, pyramidal area measuring 1 cm. at its base.

Histologic Examination

Since the vascular lesions in this case were widespread and are of primary importance, they will be described first in order to avoid repetition. The most remarkable finding was the apparently complete healing of all of the arterial lesions. Histologic examination of multiple sections from all organs failed to reveal a single vessel which was the site of active inflammation. The healed arterial lesions had been most severe and were most numerous in the kidneys, heart, liver, and mesentery, but no organ escaped some involvement. The lesions were characterized by severe intimal fibrosis, focal defects, sometimes practically complete destruction of the elastic lamellae, and fibrosis of the media, adventitia, and perivascular tissue (Figs. 4, 5, and 6).

Thrombosis was observed occasionally but was not a prominent feature except in the arteries near the hilus of the liver. Consequently, the severe intimal fibrosis was interpreted as the result of a healed inflammatory process rather than as organization of a previous thrombus. The occasional occurrence of new vascular channels and phagocytes containing hemosiderin within the intimal fibrous connective tissue suggested that obliteration of the lumen was occasionally the result of organization of a thrombus. Occasionally thrombosis was observed in a vessel which failed to reveal any abnormality in the wall. Focal disruption of elastic fibers was a particularly prominent feature of the healed lesions (Fig. 4). Aneurysm formation, however, was observed infrequently. Atrophy of muscle fibers in the media together with fibrosis was common. Extensive fibrosis of the adventitia with extension into perivascular tissues was observed also. The most significant lesion was, of course, the intimal fibrosis, which was so extensive that practically complete occlusion of the lumen occurred in many arteries.

In the right auricle there was an organizing thrombus. The muscle fibers beneath this thrombus revealed atrophy and fibrosis. Sections of the mitral valve confirmed fibrosis, vascularization, and a thrombus on the line of closure. In the aortic valve also, fibrosis and vascularization were prominent. In all sections of the myocardium there were numerous areas in which the muscle fibers were atrophic and had been replaced by hyalinized fibrous tissue. Phagocytes containing hemosiderin and lymphocytes were observed, and there were foci of recent

necrosis of muscle fibers, hemorrhage, and leukocytic reaction. On the epicardium there was an organizing fibrinous exudate.

In sections of the nodules observed in the upper lobe of the right lung there were fibrous and caseous tubercles with considerable activity as indicated by recent necrosis, epithelioid cells, and giant cells.

Sections of the liver revealed generalized atrophy of the hepatic cell columns with evidence of chronic passive congestion. Occasionally there was a focus of hyaline necrosis of the cells with accumulation of polymorphonuclear leukocytes. Sections taken through a hemorrhagic area of the liver revealed a focus of necrosis of hepatic cells surrounded by a thin layer of connective tissue and lymphocytes. Other sections revealed complete disappearance of a number of hepatic lobules with collapse of reticulum framework, fibroblastic proliferation, and preservation of bile ducts and vessels.

Sections of the duodenum revealed extensive ulceration down to the muscularis. The ulcer of the ileum also extended down to the muscularis and was covered by fibrinopurulent exudate. Fibrosis and atrophy of muscle fibers were observed in the muscularis. Epithelial elements at the edges of the ulcer revealed regenerative hyperplasia. The ulcers of the colon appeared to be of more recent origin, with hemorrhagic necrosis of the mucosa.

In the kidneys there were foci of necrosis surrounded by hemorrhages. In other sections foci of tubular degeneration were numerous. In these areas there were hyaline granular degeneration, necrosis, and sloughing of epithelial cells.

The adrenal glands were examined by Dr. W. A. Bennett, who noted atrophy of the cortex and loss of lipoid in all zones (Fig. 7). The zona glomerulosa was unusually narrow. In all zones there were many cells containing large eosinophilic granules, the significance of which was not clear.

In the testes there was severe atrophy with marked thickening of the basement membrane. Some sections revealed complete hyalinization of the tubules, whereas in others only the spermatogonia and a few spermatocytes were present in the tubular epithelium.

In the left pectoral muscle foci of hyaline necrosis and atrophy of muscle fibers with proliferation of nuclei of the sarcolemma were found. Sections of numerous lymph nodes showed atrophy of lymphoid elements with dilated lymphatic sinuses. The latter often were filled with phagocytes apparently derived from sinus endothelium.

Numerous nerves revealed focal infarcts and atrophy of nerve

trunks. In sections of the pituitary gland there was an increase in the number and size of the basophilic cells. Many eosinophilic and basophilic cells showed varying degrees of vacuolization and hydropic degeneration. Disorganization of the acinar structure and atrophy of many eosinophilic cells were present also. Sections of the brain were normal.

The following anatomical diagnoses were made: Healed periarteritis nodosa with occlusion of many arteries; old and recent infarcts of the heart, liver, intestines, kidneys, and adrenal glands; uremia (clinical); healed mitral and aortic endocarditis; healing fibrinous pericarditis; chronic ulcer of duodenum; previous (9 years, elsewhere) posterior gastrojejunostomy; chronic ulcers of jejunum, ileum, and colon; organizing thrombus of right auricular appendage; chronic fibrocaseous tuberculosis of upper lobe and healed tuberculosis of lower lobe of right lung; atrophy of adrenal glands and testes; emaciation.

CASE 2

The patient was a white man, 48 years of age, who was admitted to the hospital on September 3, 1949, with an established diagnosis of periarteritis nodosa. A duodenal ulcer had been demonstrated roentgenographically in 1941. In February, 1949, following a massive gastro-intestinal hemorrhage necessitating several blood transfusions, subtotal gastrectomy was performed.

About 6 weeks prior to admission to the Clinic, during the latter part of July, 1949, there was gradual onset of pain and weakness in his extremities, followed by fever and malaise with headache, generalized weakness, and loss of weight. These symptoms progressed rather rapidly and within 3 weeks had rendered him bedridden. On August 14 he was hospitalized elsewhere, where he remained until coming to the Clinic. A specimen of the right gastrocnemius muscle taken for biopsy on August 20 revealed lesions characteristic of periarteritis nodosa.

On admission the patient appeared acutely ill. He was mentally confused and complained of headache and of pain in his extremities. His temperature was 100.6° F.; pulse, 140; blood pressure, 174/100 mm. of Hg. Pertinent physical findings included rather marked generalized weakness with evidence of loss of weight and multiple mononeuritis involving all extremities. The retinal arterioles showed grade 1 narrowing with minimal to grade 1 sclerosis. There were a few coarse râles in the bases of both lungs, and the liver was just palpable on inspiration. Studies of the blood revealed mild anemia and hypoproteinemia. There was a 14 per cent retention of sulfobromophthalein sodium. The urine showed a specific gravity of 1.020, with albumin grade 2. A random specimen of the left gastrocnemius muscle taken for biopsy showed no definite evidence of periarteritis nodosa, although the slides of the initial specimens taken for biopsy elsewhere revealed typical lesions (Fig. 8).

During the first week in the hospital under our care the patient's condition remained essentially unchanged, with daily temperature elevations of 100° to 101° F. His leukocyte count varied between 15,900 and 25,700 per cmm. of blood, and his sedimentation rates between 111 and 113 mm. in 1 hour (Westergren method).

Administration of cortisone acetate was started on September 9, 7 days after admission to the hospital, and was continued daily for 20 days. The total dosage in

this period was 2.65 gm. and the average daily dose was 132.5 mg. There was a gradual increase in sense of well being, with clearing of the sensorium, and within 3 days the patient was afebrile. During the following 2 weeks his temperature remained normal and the pains in his extremities gradually lessened. However, there was no objective improvement of the peripheral neuropathy and both the leukocyte count and the sedimentation rate remained essentially unchanged.

On September 29, 20 days after the beginning of use of cortisone, there developed what appeared to be acute intestinal obstruction. Administration of cortisone was promptly discontinued, and with supportive measures the abdominal symptoms subsided in a few days. Administration of cortisone was resumed on October 12 and continued (except for one period of 6 days, October 25 to 30 inclusive) until January 6, 1950. The average daily dosage was 136.6 mg. and the total dosage was 11.07 gm. For both courses of cortisone the total amount administered was 13.72 gm. Except for brief periods of apparent relative remissions, the patient's clinical course during this period was characterized by increasing weakness and wasting of the muscles of his extremities with loss of weight and progressive hypertension. Tissue from the right gastrocnemius muscle taken for biopsy on October 24 (after 4.5 gm. of cortisone on 33 of 45 days) showed lesions of periarteritis nodosa (Fig. 9).

Following temporary improvement, he began to show evidence of myocardial and renal failure with reduced values for plasma chlorides, serum sodium, and potassium. Administration of cortisone was discontinued on January 6, 1950. An electrocardiogram taken on January 7 showed changes consistent with an anterior septal infarction. Funduscopic examination on January 18 disclosed an angiospastic retinopathy with hemorrhages, cotton-wool patches, and retinal edema. In addition to edema and progressive hypertension, rounding of the facial contour developed after 3 to 4 weeks of cortisone and gradually increased during the administration of cortisone. The latter gradually lessened after withdrawal of the substance. In spite of the use of digitalis and other supportive measures and attempts to correct the increasing plasma and serum electrolyte deficit, the patient became progressively worse and lapsed into coma, a terminal bronchopneumonia developed, and he died on January 27, 1950.

Necropsy

At necropsy there was generalized muscular atrophy of the trunk and extremities. Severe subcutaneous edema of the back, sacrum, and scrotum and mild edema of the ankles were present. Throughout the mesentery there were multiple nodules up to 3 mm. in diameter. The right pleural cavity contained 1 l. of clear straw-colored fluid. The pericardial cavity was obliterated by yellow, friable, fibrinous adhesions.

The heart weighed 450 gm. There were numerous, pale yellow, friable, papillary and cystic thrombi firmly adherent to the trabeculae of both atria. There was a large thrombus, 3 by 1.5 cm., in the left auricular appendage. Two nodules were observed in the coronary arteries. One was in the anterior descending branch of the left coronary artery midway between the apex and the base of the ventricle. It was 3 mm. in diameter and on section had a laminated appearance. The second nodule was situated in the right coronary artery at the

angle of origin of the posterior descending coronary branch. It was similar in size and appearance to the one on the left coronary artery. Immediately adjacent to the thoracic aorta there were similar nodules approximately 4 mm. in diameter in two intercostal arteries.

The lungs were edematous and weighed 1,745 gm. The spleen weighed 520 gm. Two hemorrhagic areas extended above the surface. On section these were seen to be large clots coextensive with hemorrhagic infarcts. The larger was 10 cm. and the smaller 5 cm. in greatest extent.

The liver weighed 2,360 gm. There was a circumscribed hemorrhagic area, 5 cm. in diameter, in the left lobe immediately beneath the capsule. Throughout the liver there were nodular enlargements of the smaller arteries. On section these nodules usually contained thrombi. Some were red and appeared recent; others appeared grayish yellow and fibrous.

The gallbladder contained numerous soft, dark brown, faceted stones.

There were two rectangular ulcerations of the mucosa of the superior portion of the esophagus which measured 2 by 1 cm. and 1.5 by 1 cm., respectively. Approximately one-half of the stomach had been resected, and the gastrojejunal stoma appeared adequate. There was an ulcer in the proximal portion of the jejunum about 2 cm. in diameter. It had a punched-out appearance with irregular borders. Its base was dark red and finely granular.

The adrenal glands appeared somewhat smaller than normal and weighed 13 gm. The right kidney weighed 165 gm. and the left, 125 gm. The surfaces were markedly irregular, with large, pitted, scarred areas alternating with nodular projections. The largest scar was approximately 2 cm. in diameter. The cut surfaces revealed pale cortices with multiple petechiae and some larger hemorrhagic areas. A small, grayish yellow, irregular, firm area was observed in the lower pole of the right kidney, surrounded by a zone of congestion. It involved both cortex and medulla and was about 1 cm. in diameter. There was a fusiform aneurysmal dilatation of two of the main branches of the right renal artery. Each was approximately 2 cm. in length by 0.8 cm. in diameter.

Examination of the brain revealed moderate external hydrocephalus. The convolutions were moderately atrophied and the sulci commensurately widened. There was a small aneurysm, approximately 8 mm. in diameter, which arose from the left middle cerebral artery and indented the left temporal lobe in the region of the uncus. An atheromatous plaque was found in the wall of the aneurysm.

Histologic Examination

The arterial lesions in this case resembled those of case 1, but thrombosis and aneurysm formation were more common (Figs. 10, 11, and 12). Aneurysms were observed in the coronary, hepatic, pancreatic, mesenteric, renal, intercostal, and cerebral arteries. Thrombosis of some arteries or arterioles was observed in practically every organ. The intimal connective tissue proliferation also noted in case 1 was characterized in some arteries by the presence of fibroblasts with multiple processes lying in a basophilic, mucinous, interstitial substance (Fig. 13). In a few arteries pronounced atrophy of the muscle fibers of the media was associated with the same type of fibroblastic proliferation. In other vessels the intimal and medial fibrosis was more advanced and was characterized by collagen fibers. In some vessels the newly formed intimal connective tissue was vascularized and occasionally was the site of hemorrhages. The media of many vessels revealed irregular fibrous scars in which the muscle fibers were atrophied or had disappeared altogether, to be replaced by fibroblasts, collagen fibrils, lymphocytes, and phagocytes containing hemosiderin. In the arteries with aneurysms there were focal disruption and disappearance of elastic and muscle fibers, the wall of the aneurysmal sac consisting only of fibrous connective tissue, presumably the remnants of the adventitia. Most of the aneurysmal sacs were filled with thrombi, often of varying ages and usually undergoing organization.

Arterioles of many organs, particularly the kidneys, liver, and pancreas, were the site of hyaline arteriolosclerosis.

Multiple sections from the myocardium revealed numerous irregular, fibrous scars. In the left auricle an organizing thrombus was adherent to the endocardium. The underlying muscle fibers appeared atrophied. Sections of the epicardium and parietal pericardium revealed an organizing fibrinous exudate.

In both lungs there were many bronchioles and adjacent alveoli filled with polymorphonuclear cells. In other sections the alveoli revealed edema, hemorrhage, phagocytes filled with hemosiderin, and plugs of fibrin undergoing organization by fibroblasts.

The capsule of the spleen was split by a large blood clot which was undergoing organization. The pulp was severely congested, and in the sinuses there were many phagocytes loaded with hemosiderin.

Examination of the liver revealed moderately severe infiltration with fat and chronic passive congestion. Sections through the capsule showed a large hemorrhage, and in the parenchyma immediately beneath the capsule entire lobules were necrotic. In other lobules hepatic

cells were in various stages of disintegration such as hyaline and granular degeneration. Many of these cells were surrounded by polymorphonuclear leukocytes.

In sections of the pancreas there were areas of parenchymal necrosis involving entire lobules and parts of lobules. In some of these necrotic lobules the ducts were spared. In other sections moderate interacinar fibrosis was observed.

Sections of the jejunum revealed ulceration of the mucosa and submucosa down to the muscular layer. Many vessels in the submucosa adjacent to the ulcer contained fibrin thrombi which were undergoing organization.

In the kidneys there were old and recent infarcts. Sections of the adrenal glands examined by Dr. W. A. Bennett were similar in appearance to those of case 1 and in addition revealed small hemorrhages in the cortex (Fig. 14).

In the thyroid gland there were recent infarcts and foci of fibrosis, and in the testis severe atrophy with absence of sperm and spermatids, and marked diminution in the number of spermatocytes.

The intercostal, pectoral, and diaphragmatic muscles showed atrophy and foci of proliferation of sarcolemmal cells. In the diaphragm there were old and recent hemorrhages and areas of fibrosis. In numerous lymph nodes there was atrophy with dilated sinuses. One mesenteric lymph node contained a focus of necrosis and small hemorrhages.

Sections of the bone marrow were not remarkable. The pituitary gland revealed vacuolization and hydropic degeneration of the eosinophilic cells with increased numbers of the basophilic cells. Some of the latter contained large vacuoles and some had a perinuclear clear zone. In sections of numerous nerves the vascular changes in the arteries were evident but there were no other abnormalities. The brain, except for a small infarct in the medulla, was normal.

The following anatomical diagnoses were made: Healed periarteritis nodosa with occlusion and aneurysm of many arteries; infarcts of heart, spleen, liver, esophagus, intestines, kidneys, and thyroid gland; uremia (clinical); mural thrombi of both atria and left auricular appendage; fibrinous pericarditis; pulmonary embolus of right upper lobe; congestion and edema of lungs with early bronchopneumonia; hydrothorax, right (1,000 cc.); atrophy of adrenal glands and testes; infiltration of liver with fat; atrophy of brain; emaciation; cholelithiasis; previous (11 months, elsewhere) gastrectomy.

Comment

The primary purpose of this communication was to record the effect, if any, of the administration of cortisone on the arterial lesions in these 2 cases of periarteritis nodosa. Although not strictly within the realm of this primary objective, certain clinical phenomena that occurred following administration of cortisone are worthy of note and may be of value in the interpretation of the morphologic alterations. These clinical phenomena may be listed as follows: (1) prompt subjective relief; (2) subsidence of fever within 24 to 72 hours; (3) a gradual decrease of elevated sedimentation rates to normal or nearly normal levels; (4) the occurrence of partial relapse after administration of cortisone was discontinued; (5) the improvement that recurred after administration of cortisone was resumed.

Histologically, the most remarkable finding was the apparently complete healing of all arterial lesions. All histologic signs of inflammation had disappeared in the first case within 3 weeks and in the second case within 3 months after biopsy had disclosed acute arteritis. Histologic examination of multiple sections from all organs in both cases failed to reveal a single vessel which was the site of active inflammation. Unfortunately, however, in the process of healing, fibrous obliteration of the lumina of these vessels occurred and resulted in infarcts which were found in many organs but were particularly numerous in the kidneys, heart, and intestinal tract.

The term healed is used here in a histologic sense to indicate complete disappearance of all signs of active inflammation, and not in the sense of restoration of pre-existing structure and function as defined by Gruber.[7] The healed arterial lesions were characterized by severe intimal fibrosis, by focal defects and sometimes complete destruction of the elastic lamellae, and by fibrosis of the media, adventitia, and perivascular tissue. Phagocytes containing hemosiderin were frequently observed in the fibrosed lesions. The intimal fibrosis which led to partial or complete occlusion of the lumen was interpreted in most instances as part of the reparative phase of the inflammatory process. Occasionally the appearance of the new connective tissue obliterating the lumina suggested that it was the end result of the organization of a thrombus.

In any interpretation of the lesions observed at necropsy, it is important to bear in mind that periarteritis nodosa is not invariably an acutely fatal disease. The course may be chronic with periods of remission and exacerbation, and rarely recovery may occur. According

to Klein,[8] spontaneous recovery may be expected under the following circumstances: confinement of the disease predominantly to nonvital structures; minimal involvement of organs followed by healing; and localization of the disease to part of a single organ. Under the latter circumstance the disease should not properly be classified as periarteritis nodosa but should be considered as focal arteritis.[9]

Cases of periarteritis nodosa verified by histologic examination in which clinical cure occurred for at least 1 year have been reported by Lindberg,[10] Harris, Lynch, and O'Hare,[11] Grant,[12] Goldman, Dickens, and Schenken,[13] MacKeith,[14] Contratto,[15] and Goodman.[16] Unfortunately, cases such as these are difficult to evaluate, since remissions and exacerbations are so often a feature of this disease. The permanence of recovery must remain uncertain unless a long follow-up study is made. Even then, clinical quiescence or remission may not necessarily indicate histologic healing. Proof of the latter cannot be established by biopsy alone because it is possible to examine thereby only superficial vessels in a small area, which may give no evidence of the disease in either an active or a healed form. Gruber [17] has emphasized that reports of recovery from periarteritis nodosa should be received with great skepticism. One is also reminded of Paltauf's [18] advice to the effect that the necropsy signifies the closure of the history of the disease and permits the final judgment concerning the favorable or unfavorable results of a morphologic process.

At necropsy one not infrequently observes evidence of healing in arteries which are the site of periarteritis nodosa (Gruber,[17] Keith and Baggenstoss,[19] Davson, Ball, and Platt [20]) in association with other arteries which are the site of acute active inflammation. Cases of periarteritis nodosa in which the diagnosis was verified by biopsy and in which necropsy later revealed no evidence of active inflammation in any of the vessels examined are exceedingly rare. Such cases have been reported, however, by Schmorl,[21] Spiro,[22] Manges and Baehr,[23] Arkin,[24] and Cathala and Boegner.[25] These cases, except for the one reported by Manges and Baehr, differ from those which are the subject of this report in that the illnesses were of much longer duration and generally lasted for a period of years. The case reported by Manges and Baehr is extremely unusual in that apparently complete spontaneous healing occurred within a period of 5½ months after a biopsy revealing acute inflammation. Pickert-Menke [26] also reported a case in which the vascular lesions were apparently completed healed. The illness from which the child succumbed lasted 1 month and 21 days, but the exact

duration of the vascular lesions is unknown since no specimens were taken for biopsy.

It should be emphasized that the histologic appearance of the healed lesions in the 2 cases which are the subject of the present report differed in no ascertainable manner from the appearance of lesions in cases described as having undergone spontaneous healing. The difference between these 2 cases and those previously reported as instances of spontaneous healing lies rather in the speed with which all signs of active inflammation disappeared. The complete disappearance of all histologic signs of inflammation, within 3 weeks in the first case and within 3 months in the second case following biopsies revealing acute arteritis, is a unique occurrence and difficult to interpret as coincidental. Atrophy of the adrenal glands in these 2 cases occurred after the administration of 3.62 gm. and 13.72 gm. of cortisone, respectively. This is consistent with known physiologic effects of cortisone, but warrants further investigation. The occurrence of atrophy of the testes and cytologic alterations in the pituitary gland is interesting, but their significance is unknown and they also demand further study.

Summary and Conclusions

In 2 cases of periarteritis nodosa cortisone acetate was administered. In the first case the patient was under our observation for 75 days and received 3.62 gm. of cortisone; in the second case the patient was under our observation for 146 days and received 13.72 gm. of the substance. Both patients were critically ill when treatment was begun. Despite initial improvement, both patients died of cardiac and renal failure.

Histologically, the most remarkable finding was the apparently complete healing of all arterial lesions. All histologic signs of inflammation had disappeared in the first case within 3 weeks and in the second case within 3 months after biopsy had disclosed acute arteritis. Histologic examination of multiple sections from all organs failed to reveal a single vessel which was the site of active inflammation. In the process of healing, however, fibrous obliteration of the lumina of these vessels occurred and resulted in infarcts, which were particularly numerous in the kidneys, heart, and intestinal tract. The healed arterial lesions were characterized by severe intimal fibrosis, by focal defects and sometimes complete destruction of the elastic lamellae, and by fibrosis of the media, adventitia, and perivascular tissue. Vascularization of the intima and phagocytes containing hemosiderin were fre-

quently observed. In the second case, the histologic findings were similar except that aneurysm formation, thrombosis, and hemorrhage were more common. In both cases, histologic examination of the suprarenal cortex revealed a moderate degree of atrophy and a decrease in lipoid content.

Although the histologic appearance of the healed lesions in these cases differed in no ascertainable manner from the appearance of lesions observed in cases in which spontaneous healing has been described, it is considered significant that the healing in these cases occurred more quickly than has ever been reported previously.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

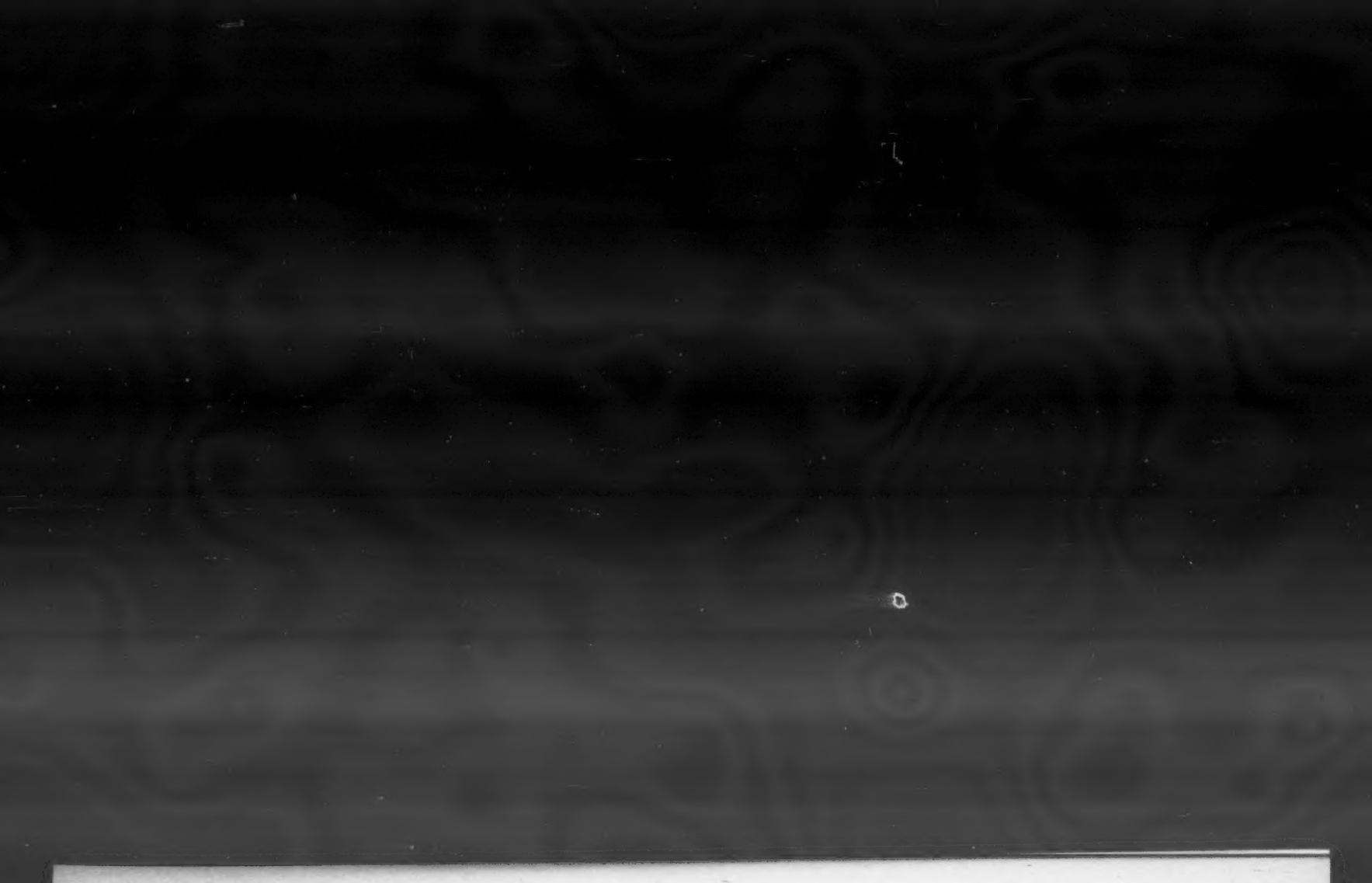
PLATE 97

FIG. 1. Case 1. Biopsy of subcutaneous nodule (June, 1949), showing acute arteritis with beginning aneurysm formation. Hematoxylin and eosin stain. × 105.

FIG. 2. Case 1. Biopsy from a painful region in the left gastrocnemius muscle (July, 1949). Acute necrotizing arteritis. Hematoxylin and eosin stain. × 55, frozen section.

FIG. 3. Case 1. Biopsy (September, 1949). Acute necrotizing arteritis 9 days after administration of cortisone was discontinued. Frozen section.

FIG. 4. Case 1. Healed periarteritis nodosa. Branch of right circumflex coronary artery, showing disruption of elastic lamellae and fibrosis of entire wall. Elastica and van Gieson's stains. × 70.



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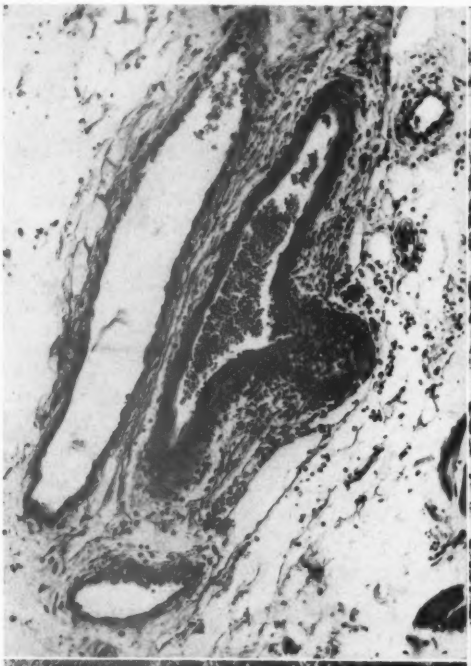

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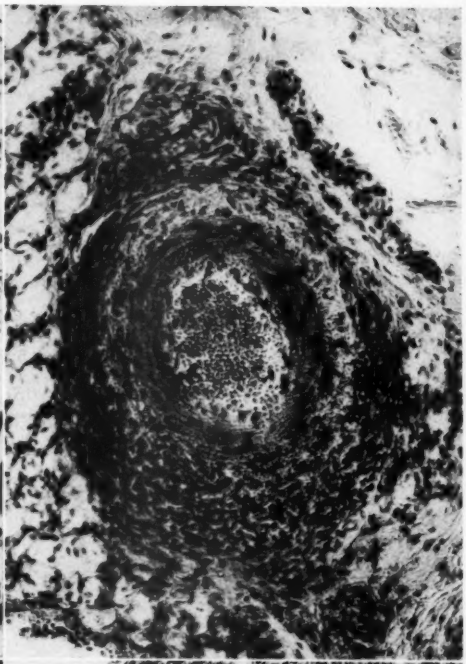

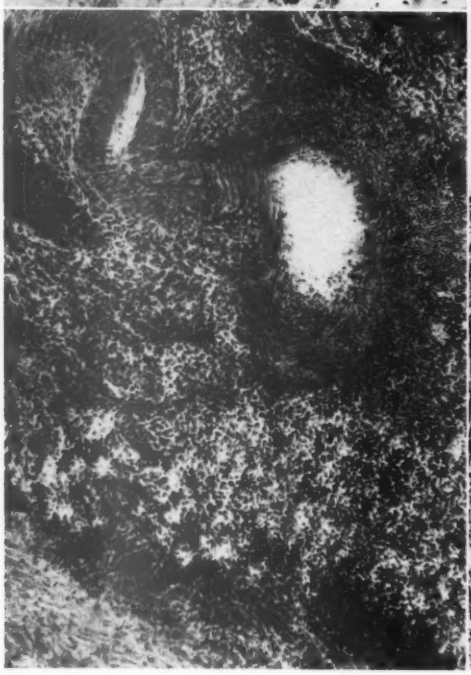

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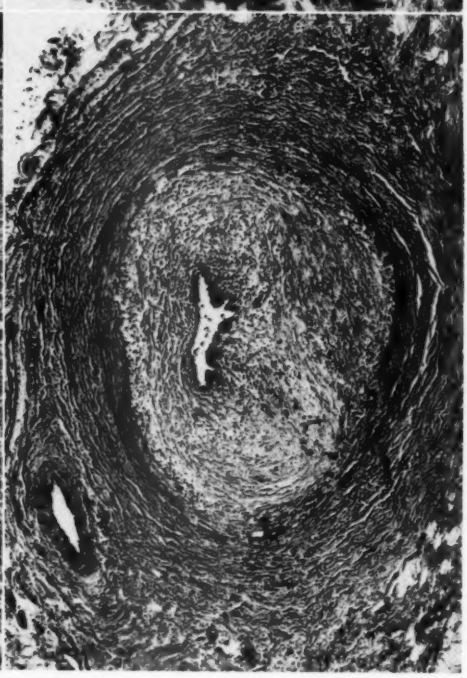

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Baggenstoss, Shick, and Polley — Cortisone on Periarteritis Nodosa

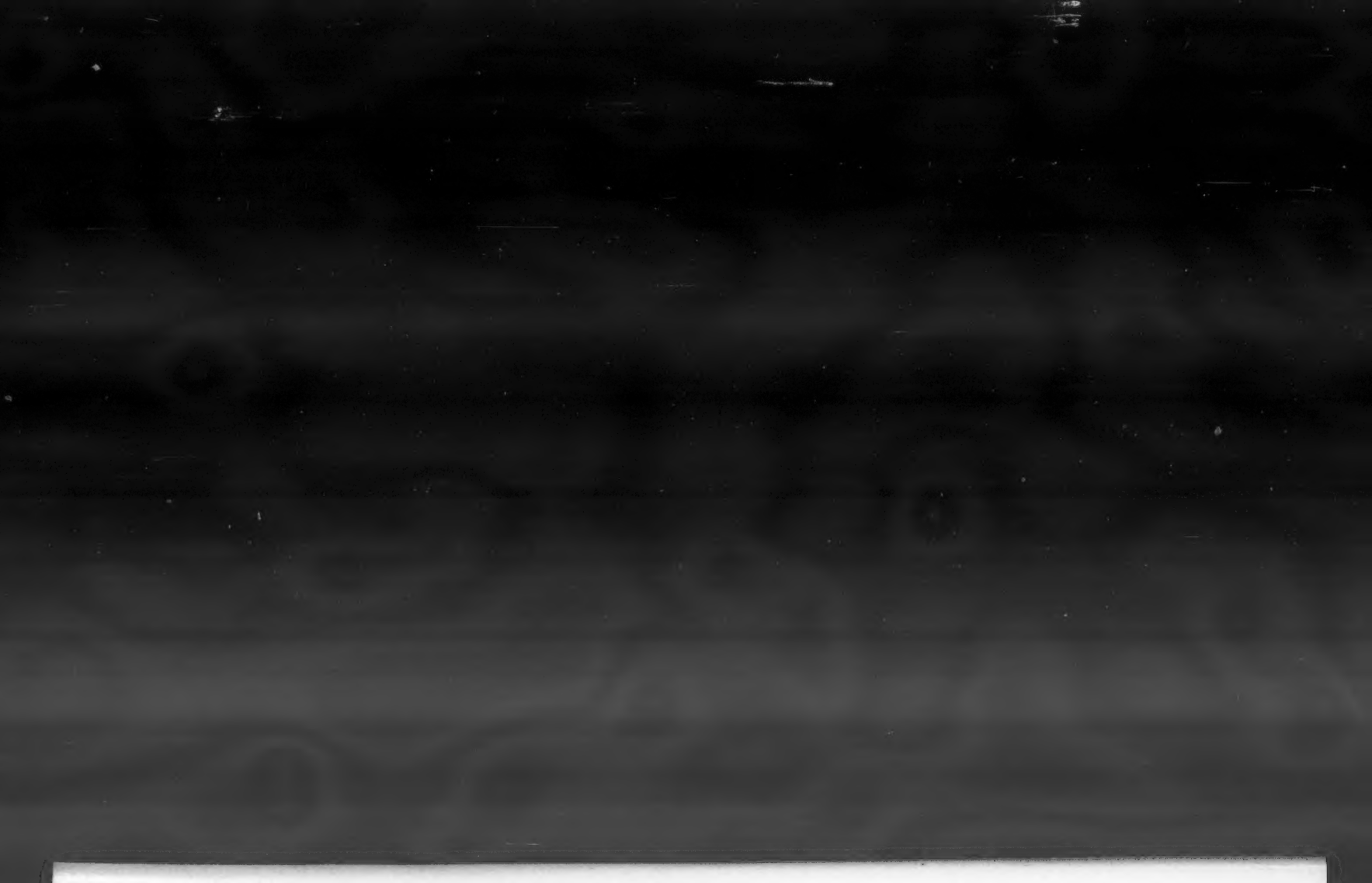
PLATE 98

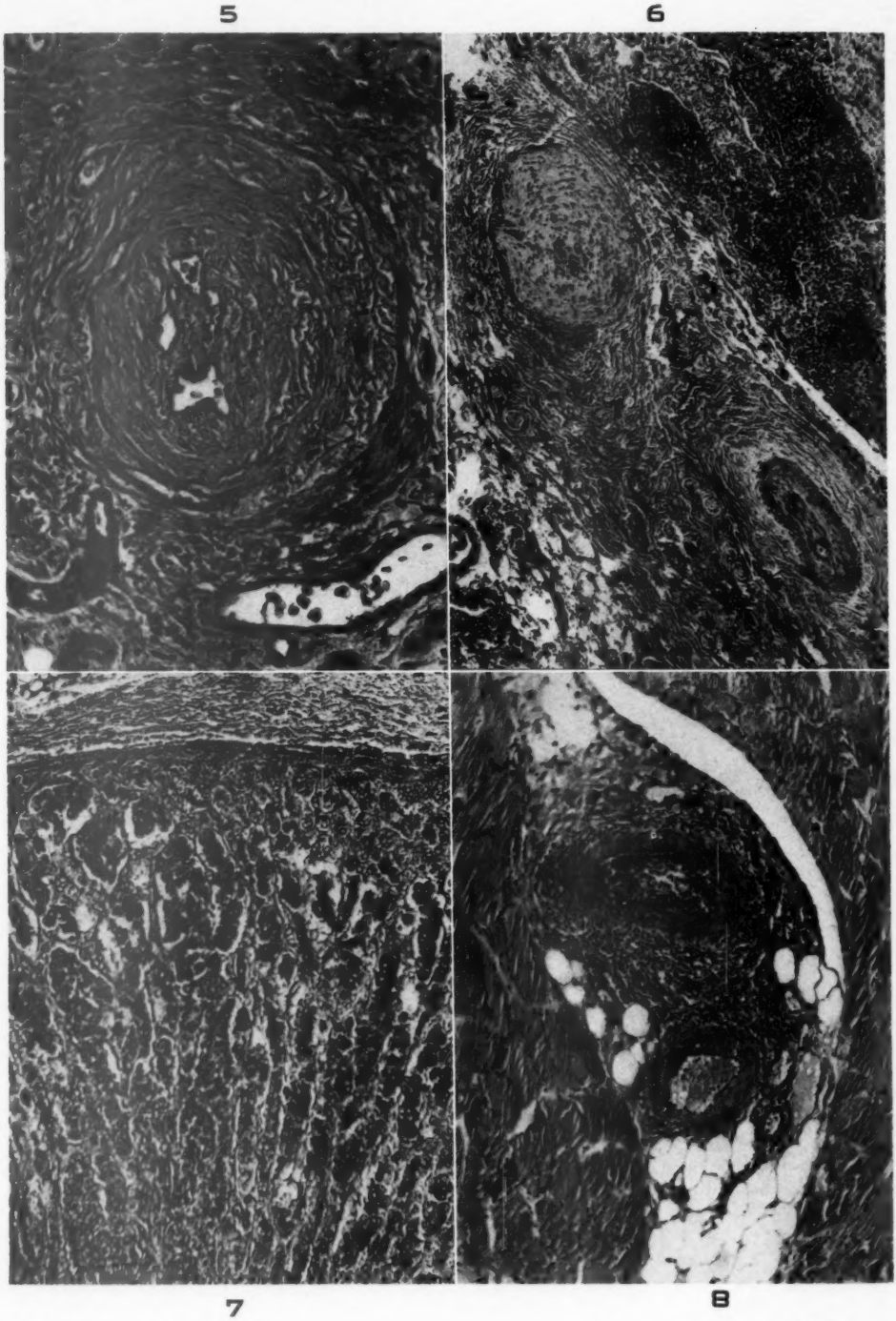
FIG. 5. Case 1. Healed periarteritis nodosa. Small artery from kidney, showing intimal fibrosis and vascularization. Hematoxylin and eosin stain. × 225.

FIG. 6. Case 1. Healed periarteritis nodosa. Branch of mesenteric artery. Fibrosis with almost complete obliteration of lumen. Hematoxylin and eosin stain. × 70.

FIG. 7. Case 1. Healed periarteritis nodosa. Adrenal gland, showing loss of lipoid and atrophy of cortical cells. Hematoxylin and eosin stain. × 135.

FIG. 8. Case 2. Biopsy of right gastrocnemius muscle (August 20, 1949). Acute arteritis with fibrinoid degeneration of the media. Hematoxylin and eosin stain. × 100.





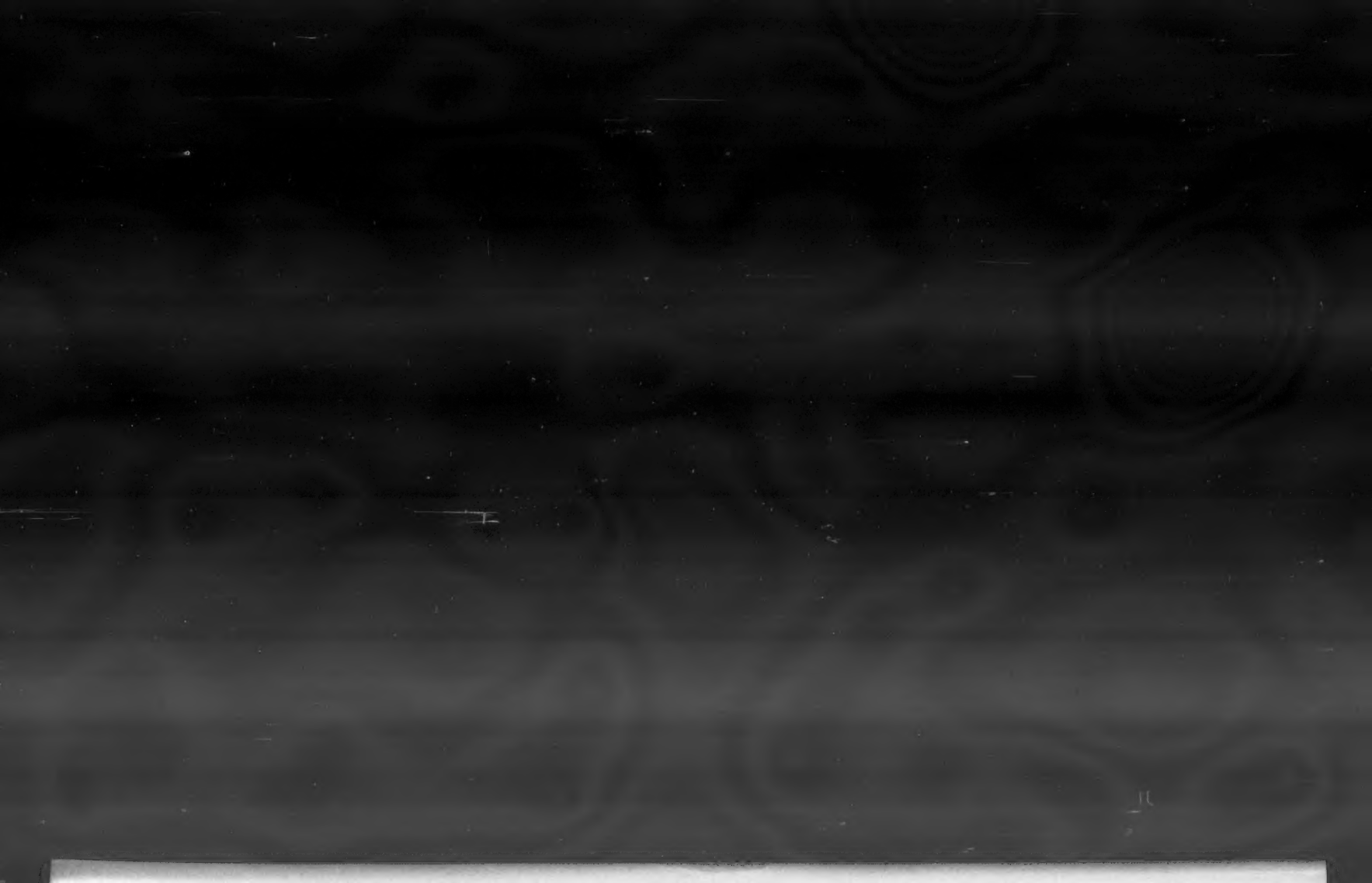
Baggenstoss, Shick, and Polley Cortisone on Periarteritis Nodosa

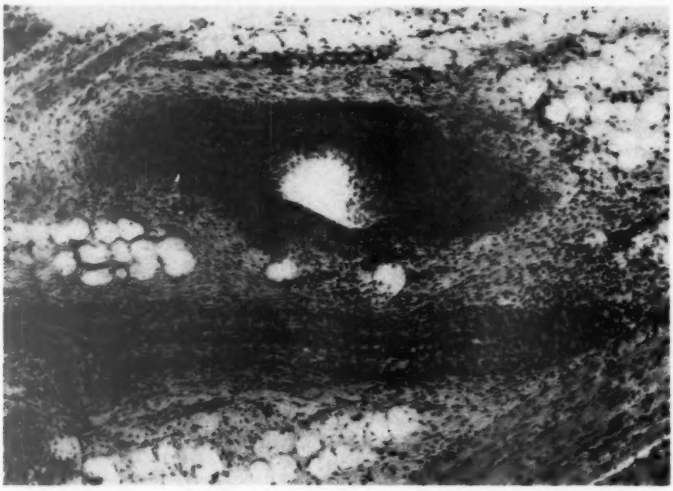
PLATE 99

FIG. 9. Case 2. Biopsy of right gastrocnemius muscle (October 24, 1949), showing active arteritis. Hematoxylin and eosin stain. × 80, frozen section.

FIG. 10. Case 2. Branch of right coronary artery, showing disruption of elastic lamellae and fibrosis of entire wall and perivascular area. Elastica and van Gieson's stains. × 45.

FIG. 11. Case 2. Small artery from kidney, with disruption of elastic lamellae, fibrosis of wall, and thrombosis with organization. Elastica and van Gieson's stains. × 42.





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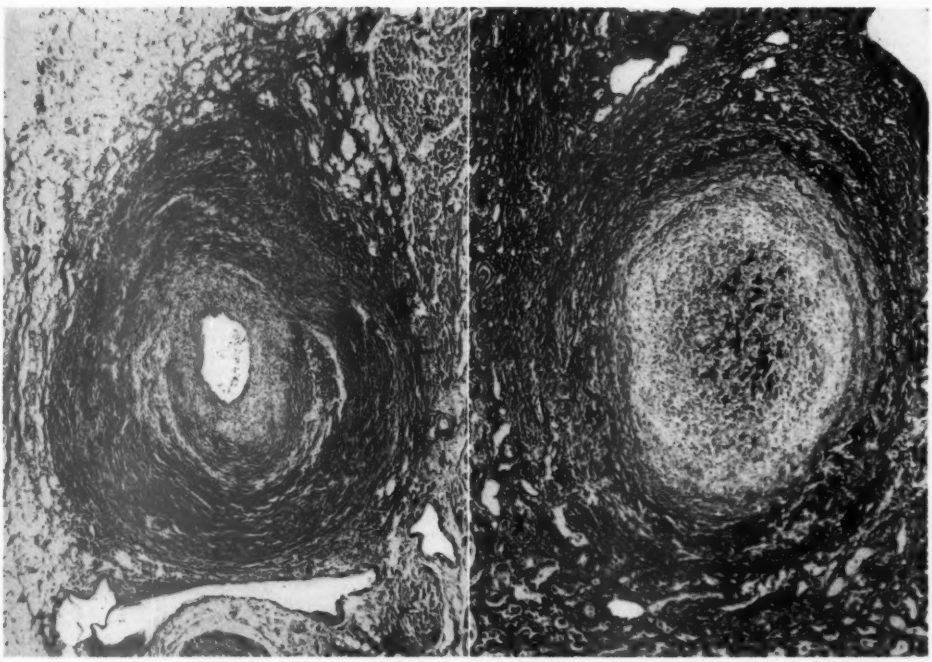

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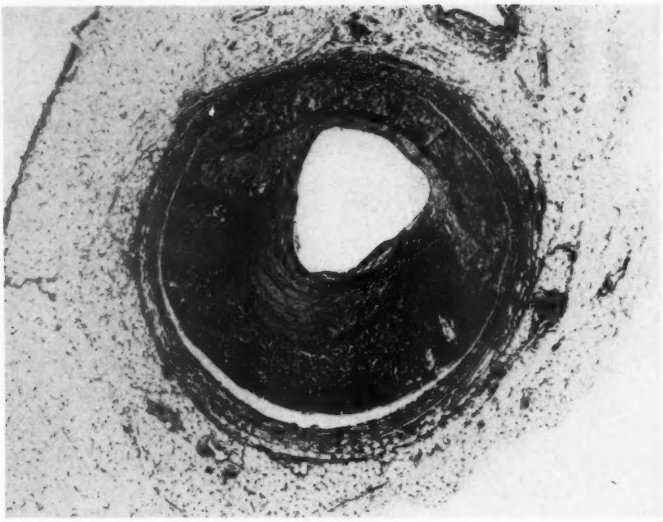
PLATE 100

FIG. 12. Case 2. Branch of mesenteric artery, showing destruction of a segment of the wall with aneurysm partly filled by thrombus. Elastica and van Gieson's stains. × 23.

FIG. 13. Case 2. Branch of mesenteric artery. Intimal connective tissue proliferation. Fibroblasts.

FIG. 14. Case 2. Adrenal gland, showing loss of lipoid and atrophy of cells of cortex. Hematoxylin and eosin stain. × 85.



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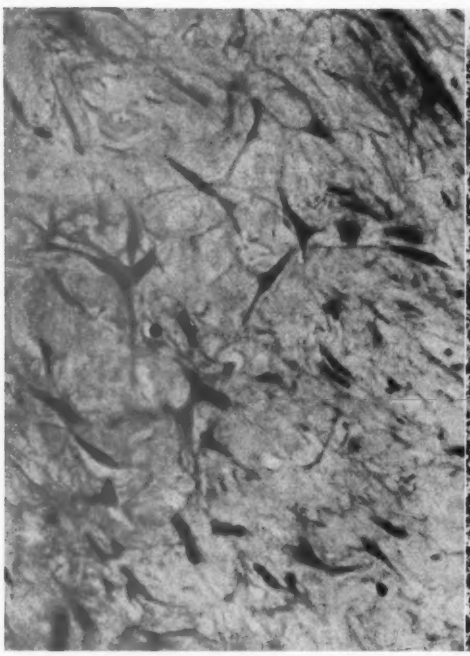

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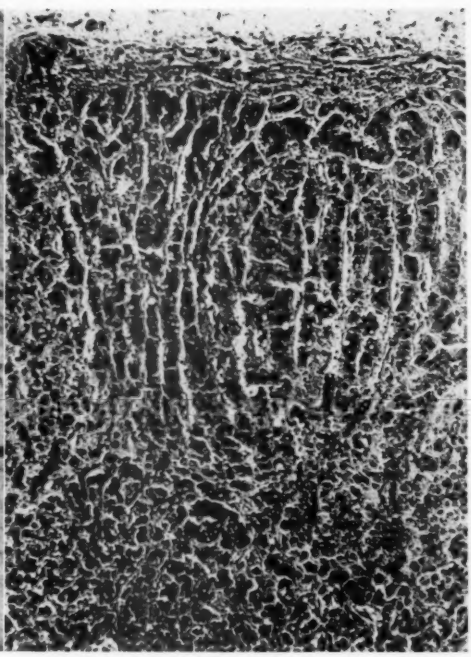

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Baggenstoss, Shick, and Polley Cortisone on Periarteritis Nodosa

"CARDIAC" OR CONGESTIVE CIRRHOSIS OF LIVER *

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Cardiac or congestive cirrhosis has long been the subject of confusion and controversy. Some seriously question the causal relationship of chronic passive congestion to the cirrhotic changes found in the liver,[1-3] while others doubt that the condition constitutes an entity. We desired further data on the pathogenesis of the lesion and its relationship to various kinds of cardiac disease. What is the extent and distribution of the hepatic fibrosis in relation to duration of cardiac decompensation? What part, if any, does mercurial intoxication play in the hepatic fibrosis that accompanies chronic cardiac failure? It seemed worth while, therefore, to review some of our necropsy records at the Los Angeles County Hospital with these questions and concepts in mind.

METHOD AND MATERIAL

A review was made of 7,075 consecutive necropsies performed at the Los Angeles County Hospital over a period of 4 years (1942–46). All cases with the diagnosis of congestive heart failure were studied. Those with all other conditions that may have an association with hepatic fibrosis were eliminated. These included alcoholism, dietary deficiencies, syphilis, chronic biliary disease, and neoplastic and parasitic diseases. There remained 605 cases, which provided our source material. From this group, those cases were selected in which clinically the patients had had congestive heart failure associated with dependent edema. Seventy-eight per cent of them had been on digitalis for a minimum of 6 months. Bouts of failure alternating with periods of compensation were the most constant clinical features encountered.

On gross examination the livers were usually smaller and firmer than the average normal organ. The surface was smooth, pebbled, or finely granular. The under surface often revealed pebbling or granularity when the upper surface was smooth. The cut surface at times showed purplish or brownish mottling due to marked chronic passive congestion but often there was little evidence of chronic congestion. The liver always cut with some increased resistance.

All original microscopic sections were reviewed. All paraffin blocks were freshly cut and stained with Mallory's aniline blue and many

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were stained by van Gieson's method. Microscopic changes of central lobular hepatic fibrosis were graded 1, 2, and 3 plus on the following basis.

Histopathologic Features

In those livers in which the fibrosis was slight (1 plus), there usually was marked atrophy of the liver cells about the central veins with dilatation of sinusoids and pooling of blood. The central vein was thickened. Reticulum of the sinusoids was definitely thickened in the central one-third to one-half of the lobule. Architecture of the lobule was only slightly disturbed (Fig. 1).

In the instances in which the hepatic fibrosis was moderate (2 plus), in addition to the fibrous network about the central veins, irregular star-shaped scars appeared in the mid- or peripheral parts of the lobules. Brush-like bands of fibrous tissue sweeping from central to peripheral areas might replace the star-shaped scars. In a few cases there was a diffuse connective tissue network throughout the lobule with some increased thickness of fibers near the center (Fig. 2).

In the more severely affected livers (3 plus), the fibrous tissue network about the center of the lobule was coarser and more of the lobule was involved than in the 2 plus group. Fibrosis in the periphery was either a network or in the form of fibrous bands. The portal spaces were usually free but might be slightly enlarged. In the more severe grades within this group the fibrous tissue network involved whole lobules or groups of lobules. Heavier bands of fibers tended to criss-cross here and there. In heavily fibrosed areas periportal spaces might be included in the fibrous changes, but in clearer areas these were free, with central fibrous networks present (Figs. 3 and 4).

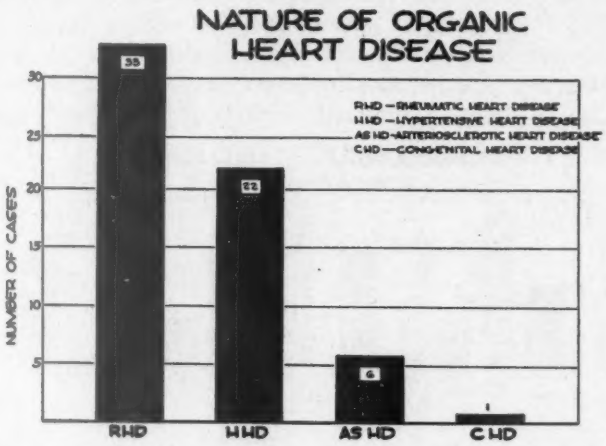
The cases designated as 1 plus showed very little disturbance of the hepatic architecture and probably should not be designated as cirrhosis. Cases classified as 2 and 3 plus showed more or less alteration of the lobular pattern due to fibrosis and may justly be classified under the heading of cirrhosis.

Results

Of the 605 instances of chronic cardiac decompensation, 62 were found that met the criteria described. Thus 10 per cent of the patients with long drawn out cardiac failure developed central and/or intralobular fibrosis of the liver. The ages of the patients varied greatly, the extremes ranging from 10 to 85 years. There was a slight increase in the number of males over females, but the ratio of males to females was the same as in the larger necropsy group.

The kind of organic heart disease resulting in congestive heart failure was tabulated (Text-fig. 1).

Rheumatic heart disease comprised 33 cases or 53.2 per cent of the whole group. It totaled 91 per cent, or 21, of the 23 cases under 50 years of age. Mitral stenosis was a finding in 23 of the 33 cases. Uncomplicated dilatation of the mitral ring was found in 4 cases. Hyper-

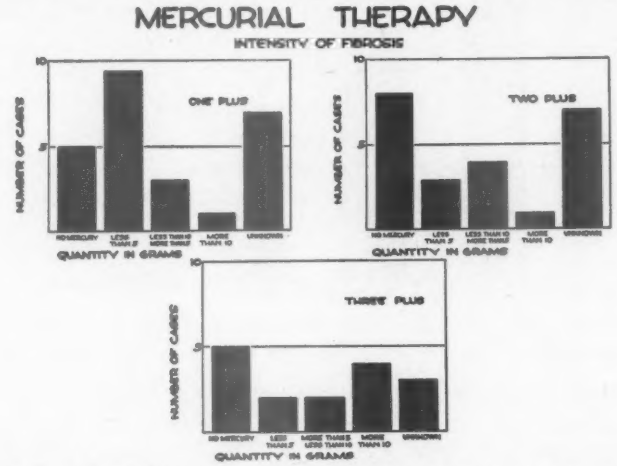


Text-figure 1. Distribution of 62 cases of congestive cirrhosis as to the type of organic heart disease present. Rheumatic cardiopathy comprised over one-half of the entire series.

tensive heart disease was the next largest group. Twenty-two patients (35.5 per cent) constituted this group. Arteriosclerotic heart disease comprised only 6 cases or 9.7 per cent. These patients presented no evidence of hypertension. A single case of congestive cirrhosis in a female, 56 years old, was noted among the 24 patients with congenital heart disease who died in congestive failure (4.17 per cent). The lesion was a large interauricular septal defect measuring 3 by 5 cm. Of the 605 patients with chronic decompensation, 7 had chronic constrictive pericarditis. Three of these developed congestive cirrhosis. Koletsky and Barnebee [4] found congestive cirrhosis present in 5 of 8 cases of chronic constrictive pericarditis. Furthermore, the fibrosis was greater in this group than in any of the other types of cardiac disease.

Butt and Simonsen [5] reported considerable quantities of mercury present in the liver and kidneys of patients dying of congestive cardiac failure when mercurial diuretics were administered over a long period of time. We have, therefore, tried to correlate the quantity of mercu-

rial diuretic administered with the degree of hepatic fibrosis in patients who received mercurial diuretics. For comparative purposes, medication has been computed as metallic mercury. A definite history of mercurial therapy was obtained in 46 of our cases. These were classified on the bases of duration of therapy and of quantity of mercury received. Seventeen patients received mercury for a period of 2 years or more; 11 patients received mercury for more than 1 but less than 2 years. The remaining 18 patients received mercury for less than 1 year. In each group based on the degree of fibrosis as 1, 2, or 3 plus, the known cases receiving mercurial therapy were arranged into groups that had (a) less than 5 gm. total mercury; (b) less than 10 gm. but



Text-figure 2. Distribution of cases of congestive cirrhosis of each of the three grades of severity as to the amount of mercury administered in mercurial diuretics. There was no positive correlation between the amount of mercury and the intensity of fibrosis.

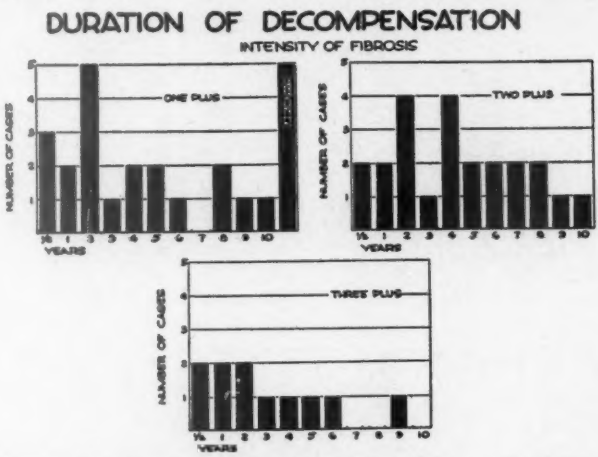
more than 5 gm., and (c) those receiving over 10 gm. The mercurial diuretics used were salyrgan-theophylline (mersalyl, U.S.P.) and mercuhydrin (N.N.R.), both of which contain 39 mg. of mercury per cc. Text-figure 2 displays the results graphically. There appears to be no correlation whatsoever between the intake of mercury and the degree of fibrosis.

Comment

From these data it is evident that hepatic fibrosis of the central lobular type may be noted as a finding subsequent to passive congestion of either short or long duration. The severity of the fibrosis, while following a loose pattern of relationship to length of decompensation and passive congestion, shows enough variation to make a definite prediction hazardous as to extent of fibrosis on the basis of duration

of cardiac failure. Cases having a history of decompensation for ½ to 10 years were observed in all grades of fibrosis (Text-fig. 3).

With rheumatic heart disease constituting the largest group (53.2 per cent) showing congestive fibrosis of the liver, and hypertensive heart disease the second (35.5 per cent), these two occur in the same order as was reported by Koletsky and Barnebee.[4] Arteriosclerotic heart disease comprised a lower percentage (9.7 per cent) than might have been expected. Seven patients in our series had chronic constrictive pericarditis but only 3 of these developed congestive cirrhosis. Others [4] have found this condition most effective in producing the more severe grades of hepatic fibrosis. There was only a single case of con-



Text-figure 3. The intensity of fibrosis in congestive cirrhosis of the liver shows an inconstant relationship to the duration of decompensation.

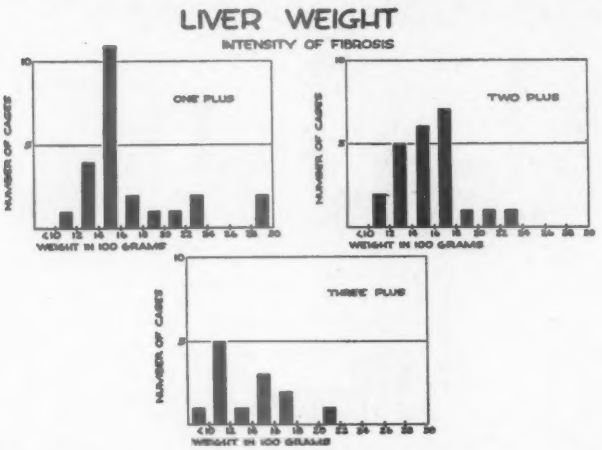
genital heart disease showing congestive cirrhosis, representing but 4.17 per cent of the total number of patients with congenital cardiac anomalies that died in congestive failure.

Wallach and Popper [6] have called attention to the central necrosis and atrophy associated with cases of congestive heart failure, emphasizing certain characteristics, such as large central veins with wide branches piercing the walls. Although there is marked atrophy of liver cells about the centers, these authors indicate that there are no fragments or remnants of cells such as are seen in acute hepatic necrosis. This probably is due to the great slowness of the process and the absence of strong cytotoxic substances. We agree with the features described by Wallach and Popper but would remark that in our cases there was little or no evidence of actual necrosis while atrophy of liver cells was a constant feature.

The fibrosis in the early cases was always more marked about the central vein or the center of the lobule. It seems to begin as a thickening of the basement membranes of the sinusoids. The sinusoids are considerably dilated and filled with blood.

Bolton [7] produced chronic passive congestion of the liver in dogs by narrowing the inferior vena cava between the liver and heart. He noted dilatation of the sinusoids with "pooling of blood" in the central parts of the lobule. He believed that stasis with consequent anoxemia was responsible for the central fibrosis thus produced. In time, increase in periportal connective tissue was noted as well.

That stasis exists in the livers of these patients with consequent



Text-figure 4. When the weights of the livers were compared with the degree of fibrosis, it was found that the liver shows progressive decrease in weight as intensity of fibrosis increases.

anoxemia is generally accepted. Anoxia of tissues in the central zones of the liver lobules is most probably the cause of liver cell atrophy and of proliferation of the more hardy connective tissues. As the process advances, in time all of the sinusoids in a given lobule may dilate, followed by increased reticular supporting tissue. With recurring bouts of cardiac decompensation over the years, spider-like scars and closely meshed networks of fibrous tissue develop, finally involving the entire intralobular parenchyma. The periportal connective tissues also are stimulated in many of the livers and probably for the same reason, a general hepatic tissue anoxia (Text-fig. 4). We are well aware that nutritional deficiencies exist in patients with chronic cardiac failure. Chronic congestion of the gastro-intestinal tract together with nausea

from digitalis therapy tends to reduce materially the food intake. If this is a factor in congestive cirrhosis, the reaction in the liver is quite different from that seen in dietary deficiencies due to alcohol.

In spite of the parenteral administration of considerable quantities of mercurial diuretics in 46 patients who had hepatic fibrosis of the congestive type, no correlation was noted between quantities of mercury given and degrees of fibrosis.

Summary

A review of 7,075 consecutive necropsies performed at the Los Angeles County Hospital during 1942–46 was undertaken as a clinicopathologic study of cardiac cirrhosis. Sixty-two cases of cardiac cirrhosis were found among 605 cases of decompensation.

Ten per cent of patients with chronic cardiac decompensation developed hepatic fibrosis. Rheumatic heart disease comprised 53.2 per cent of the 62 cases. Hypertensive heart disease was present in 35.5 per cent and arteriosclerotic heart disease in only 9.7 per cent. Three patients with hepatic fibrosis had constrictive pericarditis.

Livers showing congestive cirrhosis may be of normal size but are usually smaller than normal. The surface is often pebbled or finely granular and they cut with increased resistance. Microscopically there is often central atrophy of marked degree with a thickened reticular network in the central one-third or one-half of the lobule corresponding to the atrophied areas. In more advanced cases the fibrous network or brush-like bands occupy progressively greater amounts of the intralobular parenchyma. The central veins usually are thickened, at times to a marked degree.

The duration of cardiac decompensation varied from 4 months to 30 years and showed no constant correlation with the degree of fibrosis. Recurrent bouts of decompensation seemed to be more effective in producing hepatic fibrosis. Anoxia due to stasis associated with chronic passive congestion of the liver is considered to be the most likely cause of hepatic fibrosis. No correlation was noted between the quantity of mercury administered in mercurial diuretics and the degree of fibrosis.

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DESCRIPTION OF PLATES

PLATE 101

FIG. 1. Photomicrograph of the liver of a Caucasian male, 78 years old, showing central lobular atrophy of hepatic cells with dilated sinusoids and thickened stroma. Some central veins are obliterated, others show fibrous thickening. Spider-like spread of connective tissue from central to periportal areas (1 plus). Mallory-Heidenhain stain. × 125.

FIG. 2. Photomicrograph of the liver of a Caucasian male, 85 years of age. This shows minimal fibrosis with central atrophy of liver cells and dilatation of sinusoids. Central veins and sinusoidal basement membranes are thickened. Some periportal fibrosis is present (1 or 2 plus). Mallory-Heidenhain stain. × 125.

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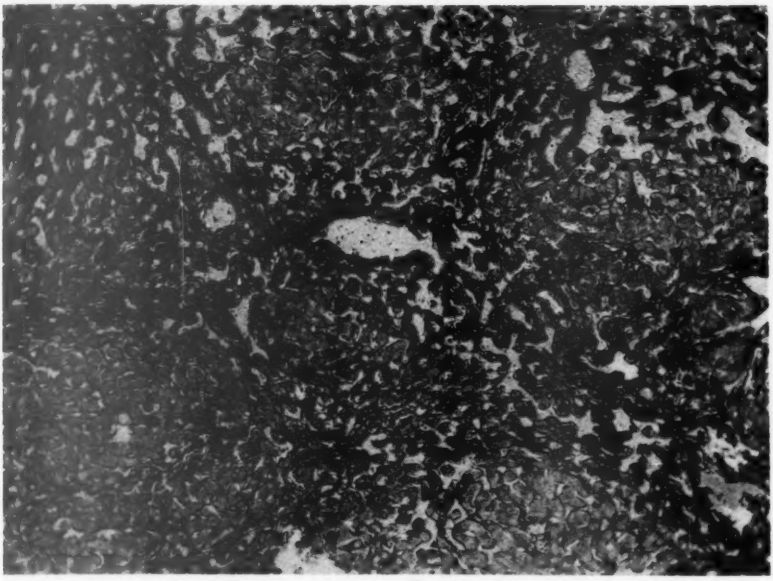

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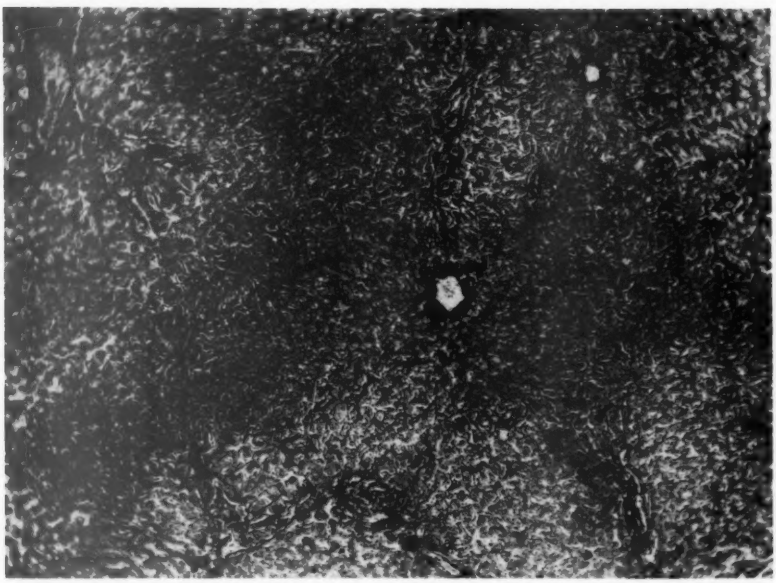

Kotin and Hall Congestive Cirrhosis of Liver

PLATE 102

FIG. 3. Photomicrograph of the liver of a Caucasian female, 39 years old. The fibrosis here is markedly increased about the central vein and throughout the lobule (3 plus). Mallory-Heidenhain stain. × 125.

FIG. 4. Photomicrograph of the liver of a Caucasian male, 16 years of age. Heavy bands of fibrous tissue pass from one lobule to another involving the intralobular hepatic tissue (3 plus). Mallory-Heidenhain stain. × 125.

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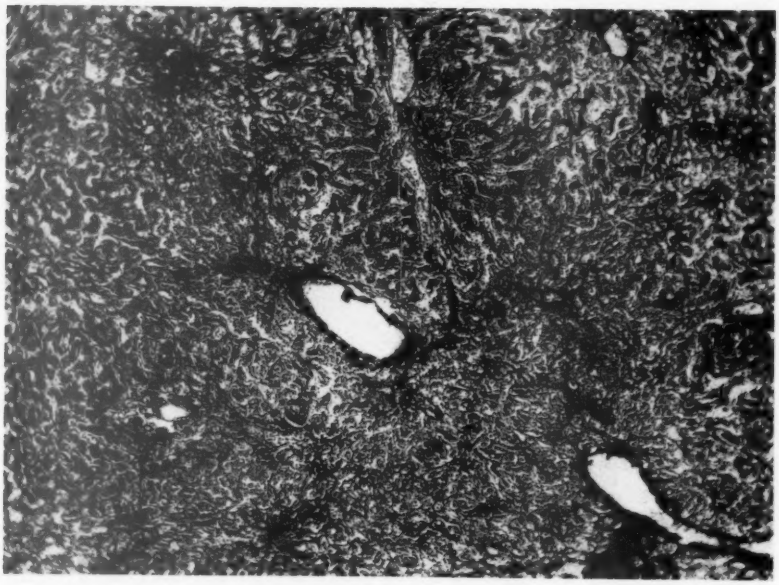

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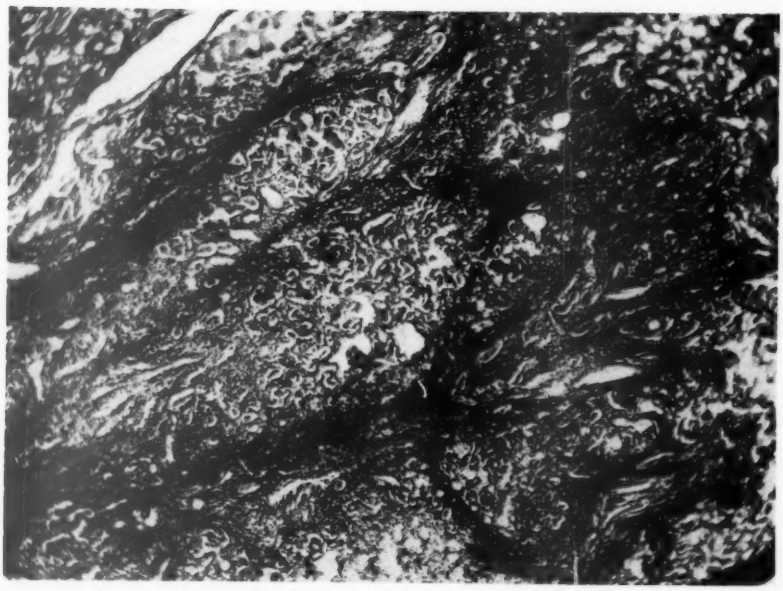

Kotin and Hall Congestive Cirrhosis of Liver

PRIMARY CARCINOMA OF THE LIVER IN HEMOCHROMATOSIS *

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Portal cirrhosis has long been recognized as a precancerous lesion. Acceptance of the disease, hemochromatosis, as a more potent factor in the causation of primary carcinoma of the liver has been contested periodically. Since diffuse nodular cirrhosis of variable degree characteristically accompanies the pigmentation in hemochromatosis, the carcinoma is assumed to arise in foci of hepatic regeneration common to cirrhosis. The existence of an increased incidence of primary carcinoma of the liver in hemochromatosis as compared to its incidence in ordinary portal cirrhosis is substantiated by slowly accumulating statistics.

Berk and Lieber,[1] in 1941, recorded primary carcinoma of the liver in 4.5 per cent of 1,989 collected cases of portal cirrhosis. In contrast, by adding to the literature 3 examples of hepatic carcinoma in a series of 15 patients with hemochromatosis, they elevated the reported incidence in this disease to 7.3 per cent. Our series contains 6 examples of primary carcinoma of the liver in 20 cases of hemochromatosis. We attempted to correlate the occurrence of carcinoma with the severity of the pigmentation and cirrhosis, and with the presence or absence of concurrent diabetes mellitus.

MATERIAL OF STUDY

Nineteen consecutive cases of hemochromatosis were obtained from the post-mortem files of the New England Deaconess Hospital, covering the period from April, 1927, to June, 1949. An additional example from the Pondville State Hospital, Walpole, Massachusetts, was the only case of hemochromatosis at this institution during the same period. The high incidence of hemochromatosis in 6,048 combined necropsies is accounted for by the large number of diabetic patients treated in the George F. Baker Clinic of the New England Deaconess Hospital. Clinical data were obtainable in all but 2 cases. Necropsies included the brain in 4 instances. Microscopic sections of organs were available for re-examination in all cases. Special stains comprised: Verhoeff's elastic tissue stain for demonstration of neoplastic invasion of blood vessels, Gomori's ferrocyanide method for hemosiderin, and Mallory's basic fuchsin for hemofuscin pigment.

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Hemochromatosis as an Entity

The picture of advanced hemochromatosis with the triad of skin pigmentation, diabetes mellitus, and hepatomegaly has held the position of an entity among idiopathic diseases since Trousseau's description [47] in 1865. The substantial group of cases with less severe pigmentation, lacking both bronzing of the skin and diabetes, was appreciated late.[2] At present, confusion exists over the distinction between hemochromatosis and visceral hemosiderosis on the basis of morphologic distribution and character of the lesions and as to etiology. The diagnosis of hemochromatosis commonly has been rejected when a known cause, such as repeated hemolysis, can be discovered.

Morphologic foundation for this attitude [3] is the predominance of distribution of hemosiderin in skin, liver, pancreas, glandular epithelium of the gastro-intestinal tract and of the endocrine glands, and choroid plexus of the brain in idiopathic hemochromatosis (Figs. 16 and 17). In contrast, hemosiderosis, as seen in hemolytic anemias, affects chiefly the reticulo-endothelial cells of the liver, spleen, and bone marrow. Clouding this simple distinction, however, are recent reports emphasizing hemosiderin pigmentation of hepatic parenchymal cells in multiple dietary deficiency,[4] ordinary portal cirrhosis,[5,6] and following multiple transfusions.[7-9] Jaffe [10] earlier mentioned this lesion in hemolytic anemias and pernicious anemia in which hemosiderin is ordinarily confined to reticulo-endothelial cells. He cited Aschoff's statement that the duration of the period of elimination of iron deposits in the liver determined their localization. Rous and Oliver,[11] by multiple transfusions of blood in rabbits, obtained hemosiderosis of parenchymal epithelium in many glandular viscera besides the liver. Present studies [12] on the fate of injected iron in animals indicate that the pattern of hemosiderin distribution is affected by the physical and chemical state of the iron, route of administration, amount of iron given, and time elapsing after injection before histologic examination.

The cirrhosis of the liver, invariably seen in hemochromatosis, has been the most reliable feature in distinguishing the typical disease from simple hemosiderosis. Yet genuine pigmentary cirrhosis, with and without hemochromatosis in other organs, has been observed at necropsy in reported cases of the following: aplastic anemia,[7,13] Banti's syndrome,[14] pernicious anemia,[13] secondary anemia of chronic glomerulonephritis,[7] agnogenic myeloid metaplasia,[8] and "pseudo-aplastic" anemia.[15,16] All of these patients had received from 1 to 39 l. of blood in transfusions. Three patients [8,13,16] had diabetes with hemosiderosis of pancreatic epithelium as well as pigmentary cirrhosis. One of

these [16] also had skin pigmentation noted clinically. These reports suggest that hematologic disorders and their treatment by blood transfusions may be important etiologic factors in certain cases of hemochromatosis, apparently by means of iron "overload." Some believe the anemic anoxia of the visceral epithelium in anemia damages the cells, predisposing them to abnormal iron retention.

Herbut and Tamaki [5] stressed the difficulty in clearly defining hemochromatosis in a study of 115 cases of cirrhosis. Ten of their series would satisfy criteria for the diagnosis of hemochromatosis. Two of this group and 10 other patients in the entire series had diabetes. The occurrence of various combinations of intracellular pancreatic and hepatic pigmentation, pancreatic fibrosis, and diabetes served to divide their cases of cirrhosis into six groups. They concluded that the difference between simple cirrhosis of the liver and genuine hemochromatosis was one of degree, and that the two extremes may be closely related.

These observations on iron distribution, etiologic possibilities, and pigmentary cirrhosis all undermine the original concept of hemochromatosis. As an example of minimal criteria for a pathologic diagnosis of hemochromatosis, we have included one case having slight hemosiderin pigmentation of hepatic and pancreatic parenchymal cells and minimal periportal fibrosis of the liver as the only evidences of the disease. If the diagnosis of hemochromatosis is admitted on these terms, the condition, while remaining a variable but distinct pathologic process, falls to the status of a syndrome clinically, with the probability of variable and possibly multiple causal factors in the individual case.

Incidence of Hepatic Carcinoma in Hemochromatosis

The largest individual series, Stewart's,[17] contained 6 instances of primary carcinoma of the liver among 52 cases of hemochromatosis, an incidence of 11.5 per cent. He compared these, together with the cases from a few small series of other authors, to a selected group of cases of portal cirrhosis from which he had deleted those with milder grades of cirrhosis. The cases with hemochromatosis were not graded. There was no significant difference in the incidence of carcinoma between those with hemochromatosis and cases with severe cirrhosis. He did not include in his statistics reports of single cases, commenting that these would have given an erroneously high figure for the incidence of carcinoma.

However, Sheldon [8] employed such reports, along with others, in his monograph and obtained an incidence of only 5.8 per cent. He collected 20 instances of carcinoma in 345 persons with hemochromatosis

(exclusive of Stewart's cases). Sheldon's figures have been widely quoted despite his own statement in the monograph that in only 119 of the 345 cases was the immediate cause of death determined "with some approach to accuracy." The number of necropsies performed in his series is not told, but an introductory paragraph states that only 311 cases were undoubted hemochromatosis.

Based on a re-evaluation of available literature, Table I includes only cases of hemochromatosis in which necropsy was performed. Be-

TABLE I

Incidence of Primary Hepatic Carcinoma in Hemochromatosis

Author	Year	No. of cases with hemochromatosis	No. of cases with carcinoma	Percentage incidence of carcinoma
Mills[45]	1924	17	3	17.6
Ophüls[46]	1926	6	1	16.6
Stewart[17]	1931	52	6	11.5
Berk and Lieber[1]	1941	15	3	20.0
Willis[40]	1941	7	3	42.9
Herbut and Tamaki[5]	1946	10	2	20.0
Author's series		20	6	30.0
	Total	127	24	18.9

cause the motives and criteria for reporting cases vary widely, we believe a complete compilation from the literature including all reports of one or several cases would be founded on erratic sampling. We have arbitrarily omitted series with less than 5 cases. Bork's group of 21 cases of hemochromatosis[18] is not statistically credited because his heterogeneous selection included examples picked from Lubarsch's handbook and from the museum of the University of Berlin. His manifest purpose was to contrast uncomplicated hemochromatosis with simple hemosiderosis. Such purposeful case selection likewise eliminates from our survey collections of examples of "exogenous hemochromatosis" in hematologic disorders. No instances of carcinoma were reported in these latter cases, some of which definitely failed to satisfy minimal criteria for hemochromatosis.

For comparison of the incidence of carcinoma in hemochromatosis with that in ordinary cirrhosis, the figures of Berk and Lieber[1] are shown in Table II. Here are added our own cases of cirrhosis occurring during the period of the study. There is a statistically significant difference in the incidence of hepatic carcinoma in the two groups. Primary carcinoma of the liver occurred in 18.9 per cent of cases of

hemochromatosis and in 4.4 per cent of cases of nonpigmentary cirrhosis.

Post-Mortem Observations

Significant findings are summarized in Table II. Data on the neoplasms are given in the case reports. To correlate the degree of pigmentation and fibrosis of liver and pancreas with the occurrence of carcinoma and severity of diabetes, respectively, pathologic involvement was estimated roughly from multiple, random histologic sections of the two organs in each case. Such grading is relative to the members of this series and has no absolute value. Examples of grades are seen in Figures 1, 2, and 13.

Table II

Incidence of Primary Hepatic Carcinoma in Cirrhosis

Author	Year	No. of cases with cirrhosis	No. of cases with carcinoma	Percentage incidence of carcinoma
Collected cases, cited by Berk and Lieber[1]	1921–1941	1039	58	5.6
Berk and Lieber[1]	1941	950	32	3.4
Authors' series		125	4	3.2
	Total	2114	94	4.4

Hepatic fibrosis was of the pattern of Laennec's cirrhosis. Increased lymphocytes were constant in the periportal connective tissue, occasionally accompanied by polymorphonuclear leukocytes. Liver pigmentation roughly paralleled the fibrosis in degree, but the severity of these lesions bore no relationship to the occurrence of carcinoma.

The direct proportion between pigmentation and fibrosis seen in most of the livers was less noticeable in the pancreas, where atrophy occasionally was as marked as fibrosis. An advanced degree of fibrosis was sometimes accompanied by only moderate pigmentation. In the pancreatic islets, pigment was invariably less in amount and involved fewer cells than in the surrounding acinar tissue. The most advanced example of islet pigmentation, together with occasional hypertrophy of islets, occurred in the absence of diabetes (case 10). Generalized fibrosis and atrophy of this pancreas equalled that found in most of the diabetic patients of this series. The patient with the most severe diabetes (case 3) had only an average degree of these changes. This paradoxic situation was stressed [19] as early as 1921 but is ignored in textbooks. Fibrosis of the pancreatic islets occurred in only 2 instances, cases 11 and 12, both with carcinoma and mild diabetes. Pancreases of 5 individuals had scanty interstitial lymphocytic infiltration.

The condition of the abdominal lymph nodes in some cases suggested that inconsistencies in visceral pigmentation might be explained by variable lymphatic drainage from the organ involved. These nodes had abundant granular deposits lying free in sinuses and within macrophages. Only case 20, however, had giant, discoid masses of siderotic material as well as the usual granular collections of hemosiderin in lymph nodes. Such unique masses (Fig. 15) were surrounded by a reaction of foreign body type with giant cells. This reaction was seen in no other instance of pigmentation of other organs or nodes in the series. The size of the masses is apparently adequate to account for the inflammatory reaction on the basis of mechanical irritation without attributing any chemical irritative property to hemosiderin. The only intracellular pigment which did not give the Prussian blue reaction with Gomori's method was in the muscle cells of the prostate in several cases, and in myocardial fibers in all cases. The myocardial pigment was typical of lipofuscin or "waste" pigment, while the prostatic pigment was stained bright red with Mallory's technic for hemofuscin.

Lesions commonly accompanying diabetes mellitus were absent or minimal in this series. Diabetic glomerulosclerosis and active pyelonephritis were not observed. The degree of arteriosclerosis was consistent with the age of the patient in every instance and in only one (case 17) was this lesion the underlying cause of death by infarction of the myocardium. Unrelated findings included a mucinous adenocarcinoma of the rectum without metastases in one patient (case 6). Another (case 17) had myeloid metaplasia in the spleen, accompanied by a slightly hyperplastic bone marrow. Among 11 cases with microscopic sections of the testes available, the germinal epithelium was absent in 4 (cases 2, 14, 16, and 20) with varying degrees of tubular hyalinization up to complete atrophy. Interstitial cells were reduced in number or absent in these 4. In one atrophy had been discovered years previously, following mumps.

Report of Illustrative Cases

Case 1

A man, 71 years old, entered the New England Deaconess Hospital on March 27, 1929, having had weakness for 2 years. Postprandial pain in the upper abdomen had been present during the 2 weeks prior to admission, accompanied by ankle edema and marked increase in generalized weakness during the latter week. On physical examination there was a hard mass palpable in the epigastrium, and edema of the legs was present. The patient was well nourished. His condition gradually deteriorated with the onset of ascites, and he died on the 62nd day in the hospital. The

clinical diagnosis was abdominal malignant neoplasm. Hemochromatosis was not considered.

Necropsy 14 hours after death disclosed a coarsely nodular liver weighing 2800 gm. through which were scattered firm, white masses of neoplastic tissue. The remaining parenchyma was red-brown, with the lobular pattern accentuated by a fibrous network. Neoplastic nodules were scattered in both lungs and upon the parietal peritoneum. Mesenteric and para-aortic lymph nodes were replaced by neoplasm. The peritoneal cavity contained 5000 cc. of clear amber fluid. The pancreas weighed 80 gm., including a moderate amount of fatty infiltration. The parenchyma was red-brown. Carcinomatosis was the cause of death.

Microscopically, the liver, pancreas, thyroid gland, adrenals, kidneys, spleen, and gastric mucosa contained moderate hemosiderin deposits. Fibrosis was moderate in the liver and pancreas.

Two forms of neoplasm were present. The majority of the liver nodules and all of the metastatic growths consisted of infiltrating sheets and closely crowded cords of cells with scanty stroma containing capillaries. Individual cells were polyhedral, similar to normal hepatic cells in size, with prominent cell membranes, and dark, acidophilic cytoplasm. Nuclei were vesicular and almost double those of hepatic cells in size. Mitotic figures and multinucleation were common. Branches of the portal vein were filled by large masses of this tissue as were small veins in pulmonary metastatic nodules. Neoplasm of a second type, occurring only in a few areas in the liver, was adenomatoid in architecture with irregular tubular and acinar formations. These acinar elements were separated by loose connective tissue, abundant in some foci but scanty in others where this neoplastic pattern merged in gradual transition with the first type. The cells of this second type were slightly smaller, and fewer mitotic figures were seen. Nuclear characteristics were similar, however, and, together with the growth pattern, distinguished these tubular groups from ordinary proliferation of bile ducts seen elsewhere (Fig. 7). Microscopic diagnosis was hemochromatosis with malignant hepatoma.

Case 5

A man, 59 years old, entered the Pondville State Hospital on June 24, 1933, with complaints of a mass of 3 months' duration and severe pain, both in the right upper quadrant of the abdomen. Pain had begun gradually 6 months previously and was aggravated by deep breathing and riding in cars. In 8 months 35 lbs. of weight were lost. Anorexia and dyspnea were more recent, lesser complaints. Physical examination disclosed moderate emaciation, clubbing of the fingers, and "cyanosis" of the

hands and feet. A hard abdominal mass extended from the fifth rib on the right downward almost to the umbilicus. Laboratory data were unavailable. General condition failed rapidly and, 2 days prior to death, purpuric areas appeared on the skin of the lower legs and abdomen. Death, preceded by coma, occurred 30 days after admission. Metastatic carcinoma of the liver, primary source undetermined, was the clinical diagnosis. Hemochromatosis was not suspected.

At necropsy, the liver, weighing 3975 gm., contained firm, yellow tumor nodules up to 1.5 cm. in diameter, scattered and confluent in both lobes. The left lobe was all but completely replaced. Non-tumorous parenchyma was marked by increased connective tissue and was dark brown. Smaller tumor nodules were present in the left kidney, lungs, thyroid gland, and adrenals. In the adrenals, 0.2 cm. was the maximum diameter of the metastatic nodules. A solitary nodule was present in the subcapsular parenchyma of the spleen and most thoracic and abdominal visceral and para-aortic lymph nodes were filled by neoplastic tissue. Implants were seen on the parietal peritoneum and the serosa of the gallbladder, small and large intestines, and stomach. Ascites, bilateral hydrothorax, and bronchopneumonia were present. The dark brown pancreas weighed 90 gm. and contained no neoplasm. The immediate cause of death was bronchopneumonia.

In microscopic sections of liver and pancreas there were hemosiderin deposits with minimal fibrosis. Thyroid gland, adrenals, and kidney were marked by similar pigmentation of less degree. The spleen contained excessive hemosiderin in the pulp. The neoplasm consisted of an infiltrating, poorly demarcated growth of large polyhedral cells arranged in elongated clusters and cords. These were accompanied by loose connective tissue, more abundant in the metastatic nodules. The cytoplasm, enclosed in a well defined cell membrane, was darkly acidophilic. Nuclei were large and, like the cell contour, varied in size and shape to a degree greater than was seen in other neoplasms in this series. Prominent nucleoli were irregular in number, size, and shape. Multinucleation was common, while mitotic figures were infrequent. Intercellular spaces existed between many of the cells in each cluster. Frequently, short processes on the cell margins vaguely suggested the intercellular bridges of stratified squamous epithelium (Fig. 12). Small areas of necrosis were numerous. Invasion of veins was seen only in the adrenal glands and lungs. The microscopic diagnosis was hemochromatosis with malignant hepatoma.

Case 11

A man, 79 years old, entered the New England Deaconess Hospital on October 1, 1940, for treatment of rectal bleeding. Diabetes mellitus had been diagnosed in November, 1939, following a history of thirst and urinary frequency for 9 months. It was readily controlled with 12 units of protamine insulin daily. Physical exam-

ination on final admission revealed emaciation, enlargement of the liver, edema of the ankles, cyanosis of the extremities, râles in both lungs, and a prolapsed hemorrhoid. Laboratory studies included: Red blood cells, 7,050,000 per cmm.; white blood cells, 11,100 per cmm.; non-protein nitrogen, 61 mg. per 100 cc. of blood; glucose, 100 mg. per 100 cc. of blood. Edema and cyanosis increased and the patient died 3 days after admission. Clinical diagnoses were myocardial failure and diabetes mellitus. Hemochromatosis and neoplasm were unsuspected.

At necropsy, the liver, weighing 1560 gm., had a finely pebbled brown surface. Roughly one-eighth of the parenchyma was replaced by friable, yellow, neoplastic nodules 3 cm. in greatest diameter and bulging at the exterior surface. Masses of neoplastic tissue partly occluded the main hepatic veins and a finger-like extension several centimeters in length protruded proximally into the inferior vena cava. The lungs contained spherical nodules similar to those in the liver. Occasional pulmonary vessels of medium size were filled with emboli of neoplastic tissue. Bronchopneumonia was present bilaterally. A single abdominal lymph node and one epididymis contained the only other metastatic nodules. About 1000 cc. of serous fluid filled the peritoneal cavity, and bilateral hydrothorax measured approximately 200 cc. on each side. Weight of the pancreas was estimated to be 75 gm., and it was brown. The immediate cause of death was bronchopneumonia.

Microscopically, hemosiderin was present in the sections of liver, pancreas, and thyroid gland to a moderate degree, while minimal amounts were contained in the adrenals, kidneys, and skin. Minimal fibrosis affected the liver and pancreas. The hepatic neoplasm was arranged in roughly spherical, well demarcated masses with scanty capillary stroma. The pattern was sheet-like, without acinar forms. Roughly 50 per cent of the neoplasm lay within the lumina of dilated veins. The cells were slightly smaller than normal hepatic cells, polyhedral, and of more uniform size than those of other carcinomas of this series. Giant nuclear forms were rare and multinucleation and mitotic figures were infrequent. Necrosis was minimal. Metastatic tumor was similar to that in the liver. Microscopic diagnosis was hemochromatosis with malignant hepatoma.

Case 12

A man, 59 years old, entered the New England Baptist Hospital on May 17, 1942, complaining of severe pain in the upper half of the abdomen. Tarry stools had been passed several times recently. Epigastric "soreness" had begun 18 months previously and was accompanied by weakness and gradual weight loss. Increase in pain was rapid during the month preceding admission. Three years previously, glycosuria was discovered incidentally, and a diagnosis of renal glycosuria was made. Two years later, in 1941, a blood sugar level after fasting of 333 mg. per 100 cc. of blood was found. The diagnosis was diabetes mellitus and 10 units of protamine insulin daily controlled glycosuria. A tender, enlarged liver, left testicular hydrocele, and systolic

apical murmur were present. The patient was poorly nourished and dehydrated. Red blood cells numbered 2,000,000 per cmm. of blood. Tarry stools continued, hematemesis appeared, and, despite seven transfusions of 500 cc. of whole blood, death occurred 7 days after entry. Clinical diagnoses were diabetes mellitus and cirrhosis of the liver with rupture of esophageal varices. Hemochromatosis and carcinoma were not suspected.

At necropsy, the liver weighed 1600 gm. and was composed of nodular brown parenchyma. The right lobe, less firm than the left, contained numerous gray-red nodules of neoplastic tissue, 3 cm. in maximum diameter and most numerous centrally. Masses of similar tissue occluded the right branch of the portal vein and many of its intrahepatic branches. The neoplasm filled many lymph nodes in the periportal, omental, and para-aortic groups, but no visceral metastases were present. The peritoneal cavity contained approximately 2000 cc. of serous fluid. The pancreas weighed about 100 gm. and was fatty. Visceral or cutaneous pigmentation was not observed. Varices, apparently intact, were present in the esophagus. The entire gastro-intestinal tract was filled with old blood. The immediate cause of death was hemorrhage.

Microscopic sections of liver contained abundant hemosiderin, accompanied by moderate cirrhosis. Pigmentation and fibrosis of the pancreas were minimal. Minute amounts of iron-containing substance were present in the adrenals and gastric mucosa. The spleen had minimal collections of hemosiderin. The liver contained carcinomatous tissue similar to the preceding case in arrangement and distribution. However, cells of two types were present. Those of one form, in the majority, resembled the cells of the preceding case in uniformity but were slightly smaller, with pale, poorly defined, scanty cytoplasm. Mitotic figures were more numerous and necrosis common. Cells of the second type were present in one section, arranged in a uniform group and similar to hepatic cells but almost twice their normal size. This mass lay within a venous lumen and was surrounded completely by neoplastic tissue of the predominating type (Figs. 5 and 6). Only cells of the smaller type occurred in the metastatic lesions. The microscopic diagnosis was hemochromatosis with malignant hepatoma.

Case 19

A man, 63 years old, who died at home on July 30, 1948, first complained of weakness and loss of weight 8 years previously. Diabetes mellitus was diagnosed then, and a maximum of 25 units of insulin daily was required for its control. One month prior to death, he entered another hospital briefly because of extreme weakness, pain in the back, and insomnia. Brown discoloration of the skin, especially of the extremities, and enlargement of the liver were observed. A needle biopsy of the liver confirmed the diagnosis of hemochromatosis. Because of rapid failure after

discharge, widespread carcinoma was suspected. Duration of his terminal illness was 1 month. Clinical diagnosis was hemochromatosis with diabetes mellitus, complicated by abdominal carcinomatosis.

At necropsy, the liver weighed 2600 gm. and had a brown "hobnail" surface, distorted in the right lobe by bulging nodules of gray-pink neoplastic tissue. The main portal vein was dilated to a diameter of 4 cm. by soft gray tissue which also filled many of its branches. Intervening parenchyma in the right lobe contained spherical neoplastic nodules. Similar masses up to 4 cm. in diameter were present in both lungs and the anterior wall of the pericardial sac was replaced by neoplastic tissue. Cervical, thoracic, and abdominal lymph nodes contained metastatic neoplasm. Several ribs with areas of softening were affected similarly. The peritoneal cavity held roughly 350 cc. of serosanguineous fluid, and hydrothorax with serous fluid measured about 1000 cc. on each side. The pancreas was average in size, brown, and without fatty infiltration. The distal two-thirds of the ileum had undergone hemorrhagic infarction, the result of thrombosis of the superior mesenteric vein. The thrombus and infarct were estimated to be roughly 12 hours old. The immediate cause of death was infarction of the ileum.

Microscopically, hemosiderin deposition and fibrosis were moderate in the liver. In the pancreas they were minimal. The adrenals, thyroid gland, kidney, and spleen contained minimal hemosiderin. The hepatic neoplasm consisted of infiltrating, poorly demarcated cords and irregular masses of polyhedral cells similar to those in case 5. Intercellular spaces here, though resembling the "mortar and brick" pattern of the latter case, had no bridging cell processes except in the metastatic nodules in the lungs. Stroma of the carcinoma varied from the scanty capillary type seen in the intravenous masses to the dense collagenous type in the adjacent invaded liver parenchyma. Mitotic figures, multinucleation, and nuclear gigantism were frequent. Foci of necrosis were numerous. The diagnosis was hemochromatosis with malignant hepatoma.

Case 20

A man, 62 years old, entered the New England Deaconess Hospital on August 3, 1948, with a diagnosis of hemochromatosis of 16 years' duration. Diabetes mellitus had been diagnosed 5 years prior to discovery of his pigmentation and subsequent positive biopsy of skin. Largest daily insulin requirement had been 48 units. Several brief episodes of diabetic acidosis had occurred in this period, and 1 month before final entry he was treated in the hospital for an initial attack of cardiac decompensation. Chief complaints on August 3 were increased weakness and anorexia. On physical examination emaciation, enlargement of liver and spleen, dusky skin coloration, moderate pitting edema of the ankles, and an apical systolic murmur were

noted. Heart sounds were faint. Laboratory data included red blood cells, 4,050,000 per cmm.; white blood cells, 11,700 per cmm. Chemical studies of the blood gave non-protein nitrogen, 42 mg. per 100 cc.; CO_2-combining power, 22 volumes per 100 cc.; glucose, 72 mg. per 100 cc. The electrocardiogram indicated an atrioventricular block. Oliguria, increasing weakness, and gradual loss of consciousness characterized the afebrile course. On the day of death, 3 days after entry, the level of non-protein nitrogen reached 93 mg. per 100 cc. of blood and the CO_2-combining power fell to 11 volumes per 100 cc. of plasma. The clinical diagnosis was hemochromatosis with diabetes mellitus. Neoplasm was unsuspected.

At necropsy, the liver weighed 1550 gm. A fibrous network divided the rust-brown parenchyma into irregular lobules producing a "hobnail" exterior surface. The posterior one-fourth of the right lobe was softened and discolored by red and purple mottling over an area which was about 10 cm. in its greatest dimension. This change penetrated to a depth of 5 cm. Section here exposed a tree-like mass of soft, gray, hemorrhagic neoplastic tissue dilating a large branch of the portal vein and extending into adjacent venous radicles for a distance of several centimeters. It was mistaken on gross examination for a simple thrombus. The main bulk of the mass was 2.5 cm. long and 0.8 cm. in diameter. No neoplastic tissue could be identified in the softened, hemorrhagic parenchyma surrounding the affected vessels. Metastases were absent. The peritoneal cavity held roughly 100 cc. of clear, yellow-brown fluid, and 1000 and 100 cc. of similar fluid were contained in the right and left pleural cavities, respectively. Sacral edema was moderate. Esophageal varices were present and intact. The weight of the pancreas was estimated to be 50 gm. The pancreas consisted of rust-brown, scanty, firm, shrunken lobules of parenchyma, widely dispersed in yellow-gray adipose tissue. The heart weighed 400 gm. Coronary arteries were patent. The immediate cause of death was congestive heart failure.

Microscopically, hemosiderin pigmentation and fibrosis were extreme in the liver and pancreas. Moderate amounts of hemosiderin marked the thyroid and parathyroid glands, adrenals, kidneys, spleen, skin, and gastric mucosa. In the liver, the neoplastic cells, arranged in broad sheets with only capillary stroma, lay almost entirely within dilated veins in the portal areas. The few parenchymal growths were sharply circumscribed. Foci of necrosis in the intravenous masses were frequent and massive. In more intact areas a tendency to disintegration of the sheets of cells produced a pseudopapillary effect (Fig. 8). Acinar patterns were absent. Arrangement and cell type were uniform throughout. The polyhedral neoplastic cells were slightly smaller than hepatic cells but had nuclei larger than those of the latter, with frequent mitotic figures. Nuclear gigantism and multinucleation

were rare. In the adjacent liver tissue, many entire lobules were necrotic, represented by pale "ghost" outlines bordered by polymorphonuclear leukocytic exudate. Occasional thrombi occluded the veins in the portal triads, and intralobular hemorrhage was frequent. The remaining liver resembled that of uncomplicated hemochromatosis. Microscopic diagnoses were hemochromatosis and malignant hepatoma with infarction of the liver.

Comment on Results of Necropsies

The outstanding feature of the gross examinations in the cases of hepatoma was the invasion of veins by the carcinoma. Preferential involvement of the right lobe of the liver and portal radicles paralleled previously reported cases. The tumors of this series were all of the multiple nodular type, in contrast to the more operable, solitary form. Metastases were more common (83.5 per cent) than would be expected from textbook accounts of primary carcinoma of the liver. Hoyne and Kernohan,[20] however, reported an incidence of metastases of 67.7 per cent in 31 patients with hepatic carcinoma, one of whom had hemochromatosis. The predilection of metastases for lungs and lymph nodes was consistent with the cases of other authors.

Microscopically, the variability of cell types and architectural patterns among the six neoplasms and within a single neoplasm was most striking (Figs. 5 to 12). The occurrence of a few foci of adenocarcinoma suggesting neoplasm of bile duct epithelium was not sufficient to classify case 1 as a cholangiohepatoma. Such features are seen in malignant hepatomas reported by others,[20,21] and adenomatoid arrangement of cells which are distinctly hepatic cells is found in examples of cholangiohepatoma [22] as well as in regeneration of hepatic lobules following necrosis.[23] Characteristics common to all members of the series included vesicular nuclei, tumor giant cells, scanty stroma, high degree of vascularity, frequent necrosis, and arrangement in cords and sheets of cells. Hemosiderin and bile pigment were absent from the tumor cells in all cases.

Attention given hemofuscin pigment in this study was minimal and we have not considered its presence to be a criterion for the diagnosis of hemochromatosis. Its uncertain identity, its disputed relationship to hemosiderin, and the disagreement over the differentiation of hemofuscin from other intracellular pigments suggest a re-evaluation of the formerly recognized pigments by newer histochemical methods. Hemofuscin is not mentioned in the recent monograph of Lemberg and Legge [24] on hematin compounds and bile pigments.

Although the immediate cause of death was carcinomatosis in only one instance in the series, indirect effects of the tumors were significant. Thrombosis of the superior mesenteric vein with infarction of the ileum, occurring in case 19, was a result of obstruction of the main portal vein by neoplasm. Scattered infarcts of the liver, secondary to tumorous obstruction of portal radicles, is also known [25] and was a contributory cause of death in case 20. In this patient, recurrent congestive heart failure in the absence of arteriosclerotic and hypertensive heart diseases requires explanation. Hemosiderosis of the myocardium with gradual hypertrophy has been the mechanism proposed in other cases of hemochromatosis.[26]

Clinical Correlation

Attempts to draw conclusions from study of the duration of the hemochromatosis were discouraged by inability to establish a time of origin of the disease. So gradual was the onset of weakness, slight pigmentation, or hepatomegaly that in one-half of our entire series a diagnosis of diabetes was the initial step in recognition. An ante-mortem diagnosis of hemochromatosis was not made in 10 of the 20 cases. More frequent use of biopsy of the liver [27] and, perhaps, studies of the utilization of radioactive iron [28,29] will probably aid diagnosis in the future. While several of our patients received blood transfusions in their terminal illnesses, there was nothing in the previous histories to suggest an external cause for hemochromatosis. Severity of the diabetes as estimated clinically by the maximum daily insulin requirement was unrelated to the occurrence of carcinoma. Two of the cancerous patients had no diabetes.

Difficulty in diagnosis of the cancerous cases was consistent with recorded experience. Other authors have commented on the variability of the clinical picture of uncomplicated primary carcinoma of the liver.[30,31] Similarity of this latter picture to hemochromatosis and to portal cirrhosis increases the difficulty of diagnosing carcinoma in the presence of either of these two diseases. Clinical diagnosis of coexisting abdominal malignant neoplasm and hemochromatosis was made in only one patient. Another patient was the only example of correct localization of the primary neoplasm clinically. In another patient, the single case without metastases, carcinoma was almost restricted to the interior of the large veins in a liver of normal size, rendering an insolvable diagnostic problem. Three of our cancerous cases had livers of normal weight. Rapid deterioration of the patient's general condition was obviously a late sign. These facts, and the frequency of abdominal pain, ascites, and weight loss in uncomplicated portal cir-

rhosis,[32] leave little in our cases to indicate hepatic carcinoma prior to the terminal illness. The poorer prognosis of carcinoma of the liver when accompanied by cirrhosis [21] also prevails when hemochromatosis is the coexisting disease. The higher grade, multinodular growth and greatly impaired hepatic reserve deny operability and hasten the course. None of this group was operated upon.

Discussion

In the accepted view of the pathogenesis of carcinoma in diffuse nodular cirrhosis, the neoplasm arises in areas of active regeneration or hyperplasia of hepatic cells.[30,33,34] Since these foci of cellular activity are present in hemochromatosis (Figs. 3 and 4), the mechanism of carcinomatous transformation may be the same. The difficulty of histologically differentiating these bizarre foci of regeneration from areas of neoplasm in cirrhosis or hemochromatosis [35] gives morphologic support to Orth's original concept (1887) as phrased by Muir,[34] that the hyperplasia simply exceeds normal bounds and becomes neoplastic. A corollary, that carcinoma is most likely to arise in the livers showing the most "pronounced" hyperplasia, further emphasized this aspect.[36] Since hepatoma in portal cirrhosis occurs chiefly in the sixth decade, at a time when the cirrhosis is usually well advanced, some have concluded that parenchymal hyperplasia varies directly with the severity of the cirrhosis. Our studies do not support this reasoning. Two of the six hepatomas occurred in the presence of the lowest grade of fibrosis (Fig. 1) while other non-cancerous examples in the series had marked pigmentary cirrhosis (Table III). The advance of cirrhosis, we believe, represents a quantitative failure of regenerative activity, a lessening of the total amount of hyperplasia continuing in the liver. The fibrosis is not a direct result of hepatic injury and is absent after injury when multiplication of hepatic cells is sufficient to keep pace with destruction. If reparative activity lags, stroma collapses and cirrhosis appears, as evidence of deficient regeneration of hepatic cells. The peculiar foci of new cells in Figures 3 and 4 indicate handicapped regeneration instead of the more orderly, abundant, lobular reproduction seen in experiments using simple partial hepatectomy in normal dogs.[37] Regenerative foci in cirrhosis or hemochromatosis must contend with impaired blood supply, stromal collapse with fibrous tissue competition, and probable persistence of an etiologic factor. The cellular changes recall the epithelial phenomena in chronic thyroiditis, pyelonephritis, and peptic ulcer.

Although we believe the carcinoma arises in hyperplastic centers, factors other than total regenerative activity must be important. Those

factors would condition the regenerative process qualitatively and, if known, might explain why only certain cases of prolonged hyperplasia of hepatic cells terminate in carcinoma. Experimentally, special properties of the noxious agent have been of obvious importance in the production of hepatomas without cirrhosis in rats fed aminoazotoluene for over 7 months. In this work of Yoshida (cited by Shear [88]),

TABLE III

Clinical and Pathologic Data on Twenty Cases of Hemochromatosis

Case no.	Age	Weight of liver	Weight of spleen	Grade* of fibrosis in liver	Grade of pigmentation in liver	Grade of fibrosis in pancreas	Grade of pigmentation in pancreas	Maximum daily insulin requirement	Immediate cause of death
		gm.	*gm.*						
1†	71	2800	190	2+	2+	1+	2+	N.D.§	Carcinoma of liver
2	59	2170	200	1+	2+	1+	1+	30 U	Lobar pneumonia
3	54	2500	275	2+	3+	2+	3+	114 U	Cardiac failure
4	55	2680	325	2+	3+	1+	3+	30 U	Peritonitis
5†	59	3975	275	1+	2+	1+	2+	N.D.	Broncho-pneumonia
6	70	"Normal"	200	1+	1+	0	1+	N.D.	Broncho-pneumonia
7	43	2000	240	3+	1+	1+	2+	56 U	Cardiac failure
8	58	1810	110	2+	2+	1+	3+	21 U	Hemorrhage from esophageal varix
9	42	"Moderate enlargement"	"3× normal"	2+	2+	2+	3+	23 U	Pericarditis
10	70	1380	240	2+	2+	2+	3+	N.D.	Broncho-pneumonia
11†	79	1560	140	1+	2+	2+	2+	14 U	Broncho-pneumonia
12†	59	1600	200	2+	3+	1+	1+	20 U	Hemorrhage from esophageal varix
13	58	880	260	2+	1+	2+	F.T.‡	12 U	Broncho-pneumonia
14	55	1240	690	4+	2+	1+	1+	N.D.	Broncho-pneumonia
15	80	500	150	1+	1+	2+	1+	No data	Cardiac failure, pulmonary edema
16	53	2940	850	3+	3+	2+	4+	80 U	Cardiac failure, congestive
17	49	2260	460	3+	3+	2+	1+	36 U	Myocardial infarction
18	42	1075	285	2+	2+	1+	1+	Diet only	Hemorrhage into g.i. tract
19†	63	2600	480	2+	2+	1+	1+	25 U	Mesenteric thrombosis with infarct of ileum
20†	62	1550	250	4+	3+	4+	2+	48 U	Cardiac failure, congestive

* Relative grades from 1+ to 4+, referred to in text as 1 to 4.
† With primary carcinoma of the liver.
‡ Faint trace of pigment present.
§ No diabetes present.

active regeneration became more atypical histologically until it progressed to carcinoma. Nelson, Fitzhugh, and Calvery[39] saw severe cirrhosis, followed by occasional low-grade hepatomas in rats fed selenium for over 18 months. They concluded that ageing of the animals may have been the deciding factor and noted no parallelism between the severity of the cirrhosis and occurrence of the neoplasm. Age may be an important factor in carcinogenesis in hemochromatosis. The average age of our patients with carcinoma was 65.5 years as compared to 56.3 years in the noncancerous group. In the series of Willis[40] (the only author who reported ages completely), these figures were 64.3 years and 51.5 years, respectively.

The problem of enhanced carcinogenesis here is doubtless linked to the cause or causes of hemochromatosis. The minority of observers,[3] focussing attention on the pigmentation, believed that the iron deposits represented a primary, "inborn," perhaps familial, metabolic disorder and caused death of epithelial cells in the liver and pancreas. Few, however, credited the pigmentation as the cause of the fibrosis in either liver or pancreas. Other investigators, struck with the combination of cirrhosis and diabetes, postulated an acquired metabolic or toxic damage to the liver and pancreas, resulting in cirrhosis and, often in diabetes.[11,41,42] They regarded the pigmentation as secondary or incidental to the underlying disease. Whether the source of the excessive hemosiderin is in increased intestinal absorption [7,28] (idiopathic type) or in intravenous injection of whole blood,[7] its significance in the epithelial cell is practically unknown. Precipitated in secretory cells which are presumably previously damaged, the iron excess undoubtedly contributes to death of the cell. The idea that hemosiderin deposits in hemochromatosis might predispose to carcinoma is not new.[43] Direct evidence is lacking, however. Campbell,[44] obtaining an increased incidence of pulmonary carcinoma in rats which inhaled pure ferric oxide dust, may have provided a clue. Exploration of the intercellular metabolism of iron must be extended in order to apply such observations to our problem. Together, primary progressive parenchymal damage to the liver and subsequent deposition of iron in hepatic cells seem to provide a setting for carcinogenesis more effective than simple portal cirrhosis.

Conclusions and Summary

Six cases of primary carcinoma of the liver occurred in a series of 20 consecutive patients with hemochromatosis. These cases raise the reported incidence of hepatic carcinoma in hemochromatosis to 18.9 per cent. This figure includes only those series from the literature

having 5 or more consecutive cases of hemochromatosis upon which necropsies were performed.

No correlation existed in this series between the duration or severity of hemochromatosis as estimated anatomically, and the occurrence of carcinoma.

No correlation was found between the presence, duration, or severity of the diabetes and the occurrence of carcinoma.

The average age of our patients with uncomplicated hemochromatosis was 56.3 years; those with carcinoma had an average age of 65.5 years.

The factors responsible for the increased incidence of primary carcinoma of the liver in hemochromatosis as compared to that in portal cirrhosis remain unidentified.

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DESCRIPTION OF PLATES

PLATE 103

FIG. 1. Case 11. An example of grade 1 pigmentary and fibrotic involvement of the liver in a patient with malignant hepatoma. Neoplasm not present in this area. × 50.

FIG. 2. Case 20. Grade 4 lesions of pigmentation and fibrosis in a patient with malignant hepatoma. Pigment is noticeable in bile duct epithelium as well as in hepatic cells. Neoplasm not present in this area. × 50.

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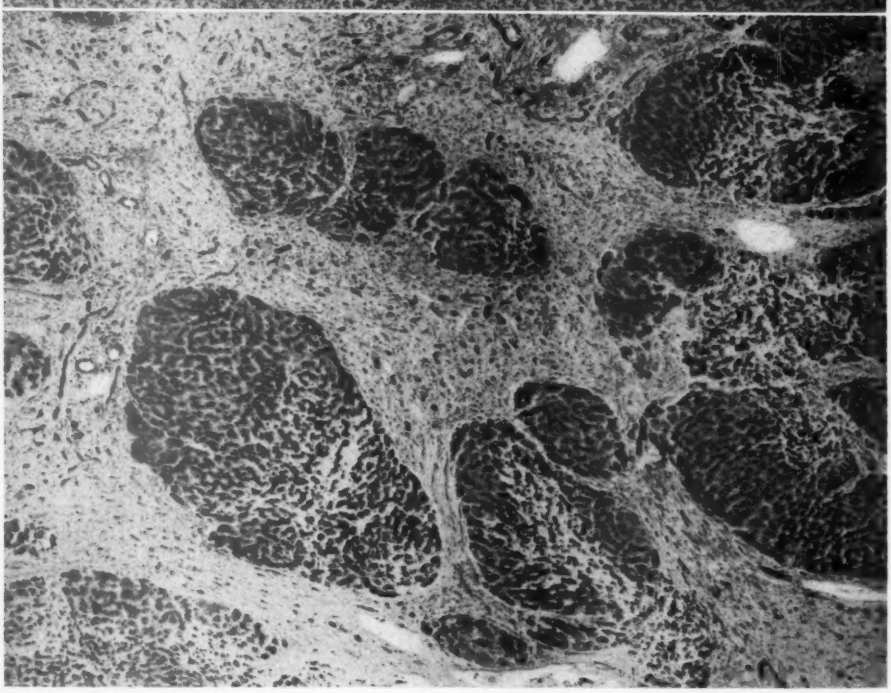
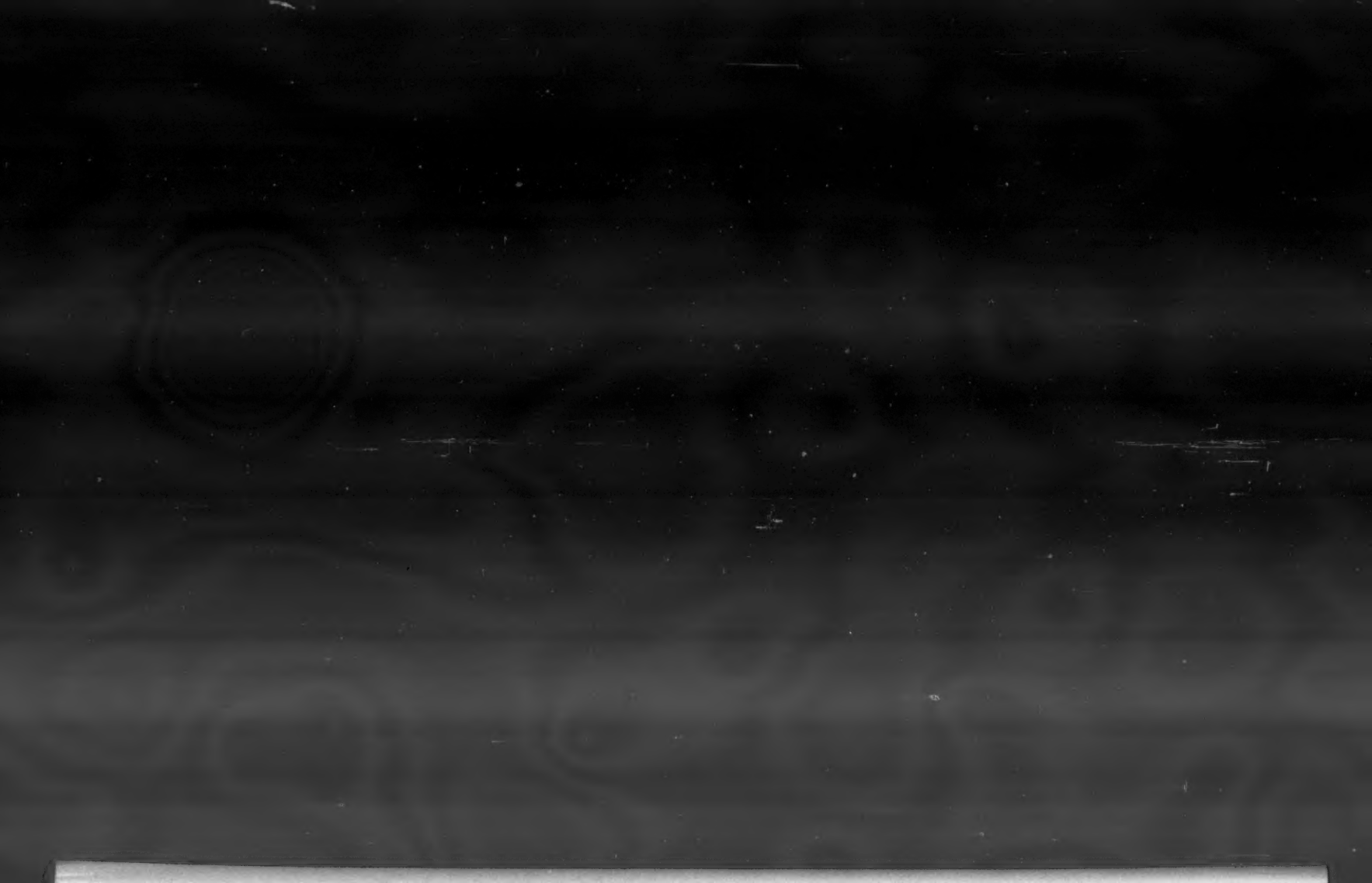


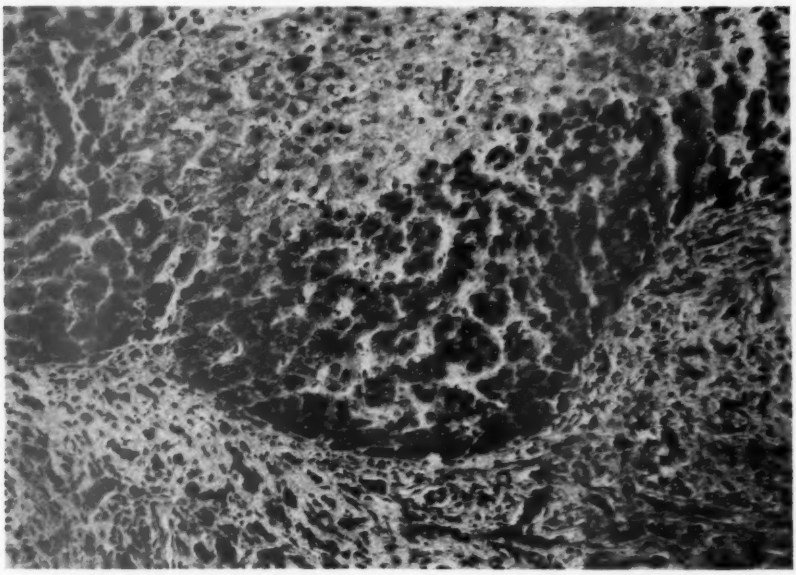
Warren and Drake Primary Hepatic Carcinoma in Hemochromatosis

PLATE 104

FIG. 3. Case 20. A focus of hepatic cell regeneration (upper center) in a liver lobule. The nuclei are enlarged and pigment is almost absent in contrast to remainder of lobule. Eosin and methylene blue stain. × 125.

FIG. 4. Case 20. Area of regeneration in center of field, similar to that in Figure 3. Such foci are supposed sites of origin of neoplasm. Ferrocyanide stain, iron hematoxylin counterstain. × 125.





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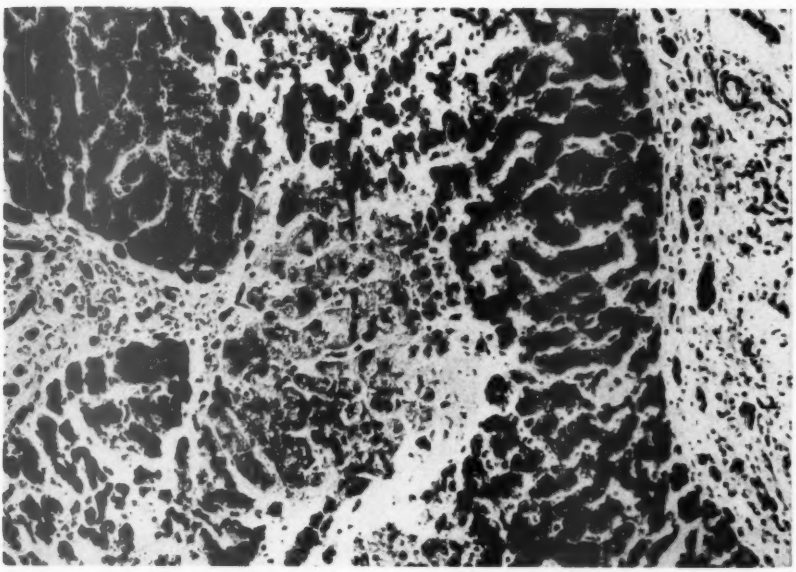

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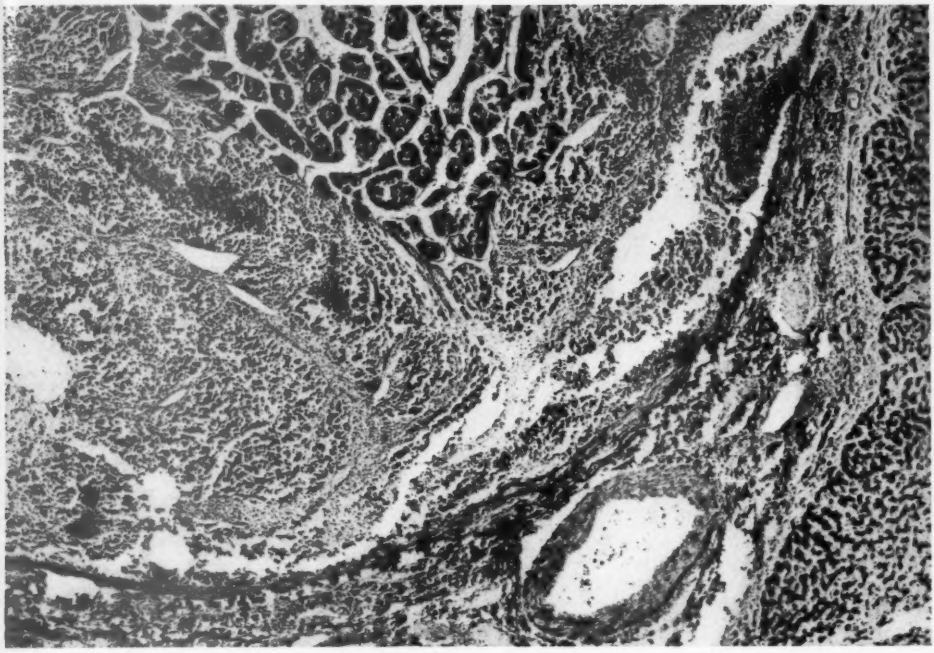
PLATE 105

FIG. 5. Case 12. Multiform neoplasm lying within the lumen of a vein. The lumen occupies the entire upper left portion of the field, the thin arc being the wall of the vein. In the upper central portion is carcinoma of the large cell type, surrounded by neoplasm of the small cell type. × 45.

FIG. 6. Case 12. Enlargement of the neoplastic field in Figure 5. Resemblance of large tumor cells to normal hepatic ceils is remarkable. Despite high vascularity, several foci of necrosis are present in the field of small tumor cells. × 105.



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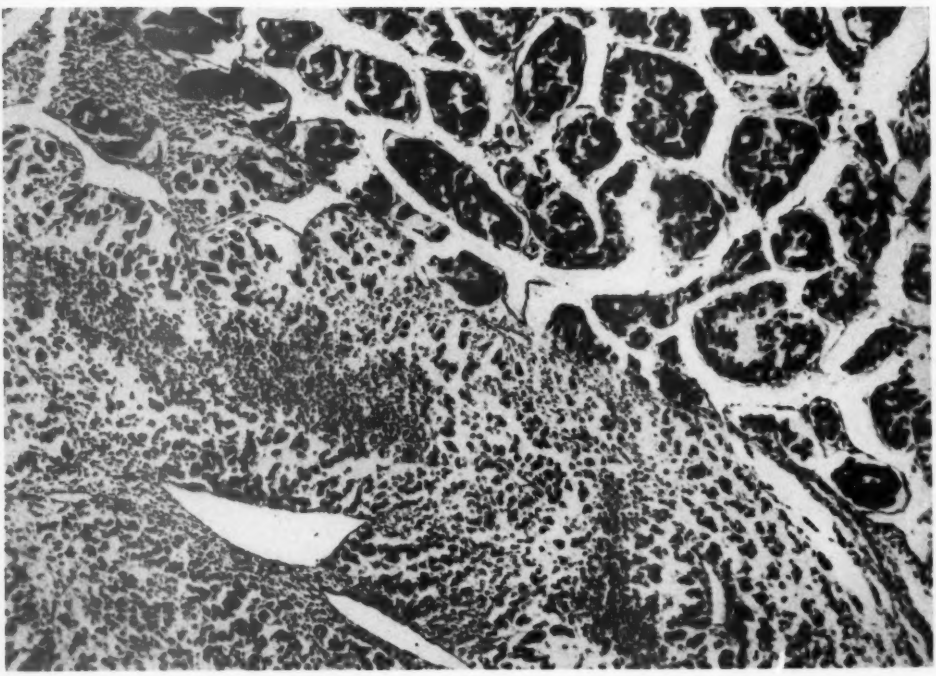

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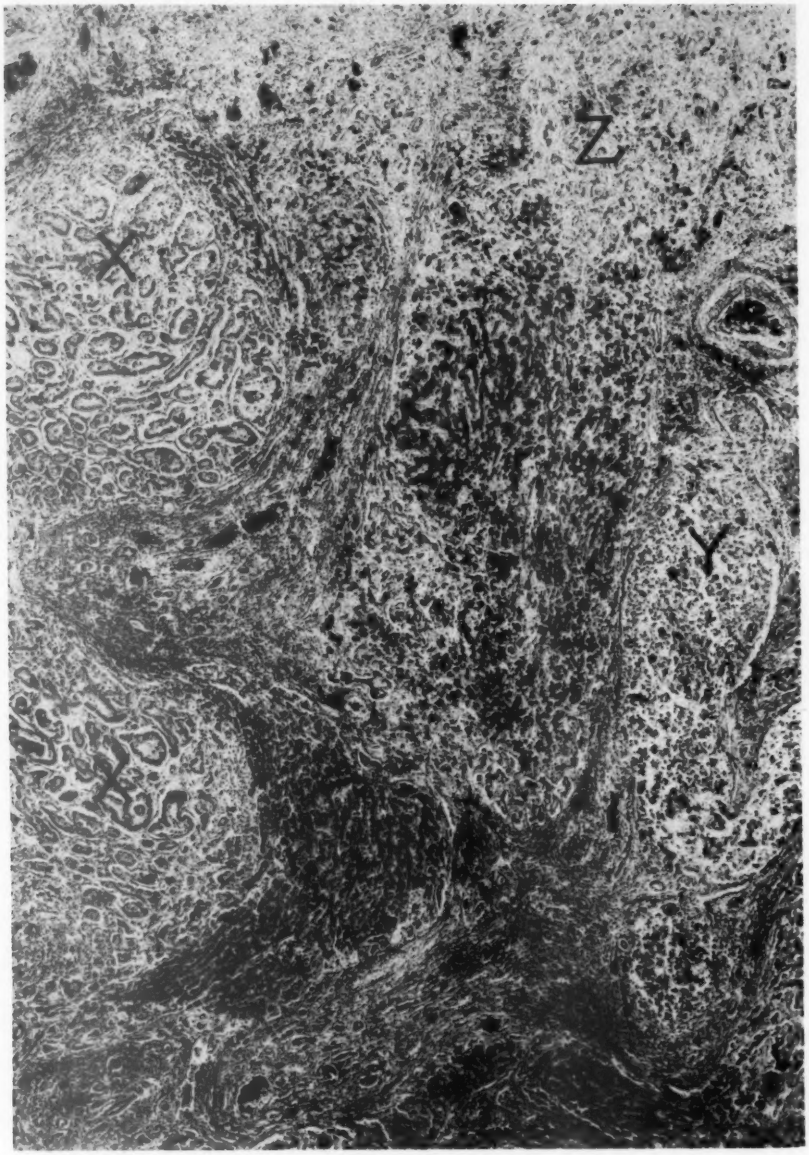
Warren and Drake Primary Hepatic Carcinoma in Hemochromatosis

PLATE 106

FIG. 7. Case 1. Multiform neoplasm growing as adenocarcinoma at X, and in simplex pattern within vein at Y. Other fields contained only the simplex form. Proliferating bile ducts at Z have smaller cells than the neoplasm at X. × 50.



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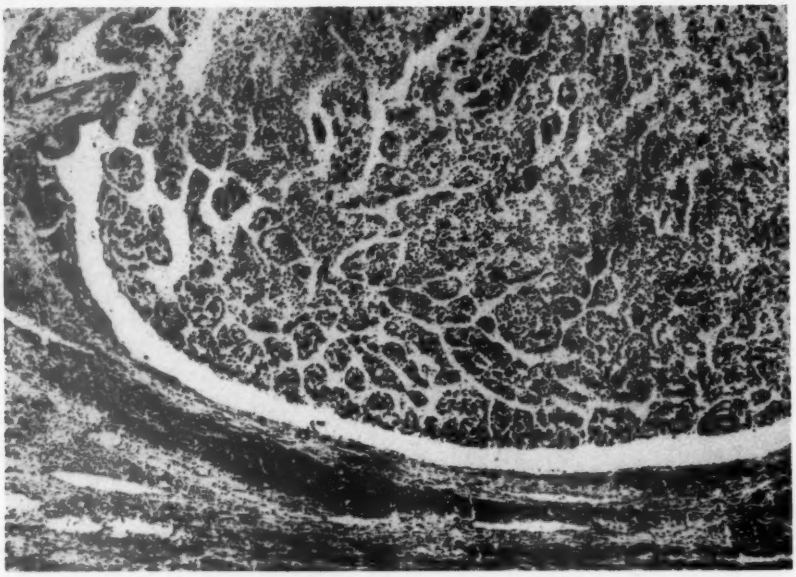
Warren and Drake Primary Hepatic Carcinoma in Hemochromatosis

PLATE 107

FIG. 8. Case 20. Malignant hepatoma growing within lumen of portal branch. Degeneration and disintegration create the pseudo-papillary pattern described by Winternitz. × 50.

FIG. 9. Case 11. Neoplasm with unusual regularity of cells. Nucleoli are prominent with the eosin and methylene blue stain. × 250.



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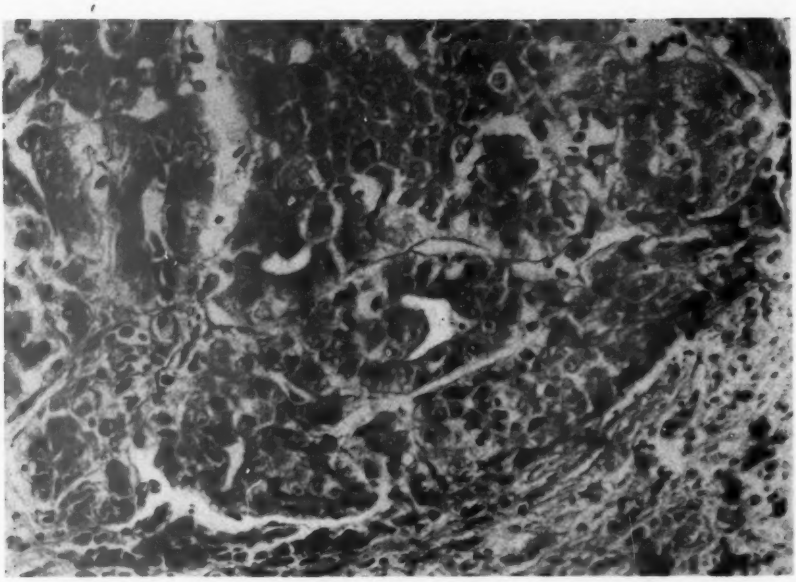

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PLATE 108

FIG. 10. Case 1. Typical permeation of a portal branch by carcinoma of simplex pattern. The center is necrotic. Same case as Figure 7. Pigmentation and fibrosis of grade 2 were present in areas of parenchyma away from tumor. × 125.

FIG. 11. Case 5. Malignant hepatoma invading cords of liver cells. Anaplasia and infiltrative tendencies were greater than in other tumors of the series. Formation of abortive cords is apparent and cytoplasmic membranes are often distinct. Moderately pigmented hepatic cells form normal cords (lower right); others, mistaken for pigmented neoplastic cells, are scattered (upper left). Phosphotungstic acid hematoxylin stain. × 500.

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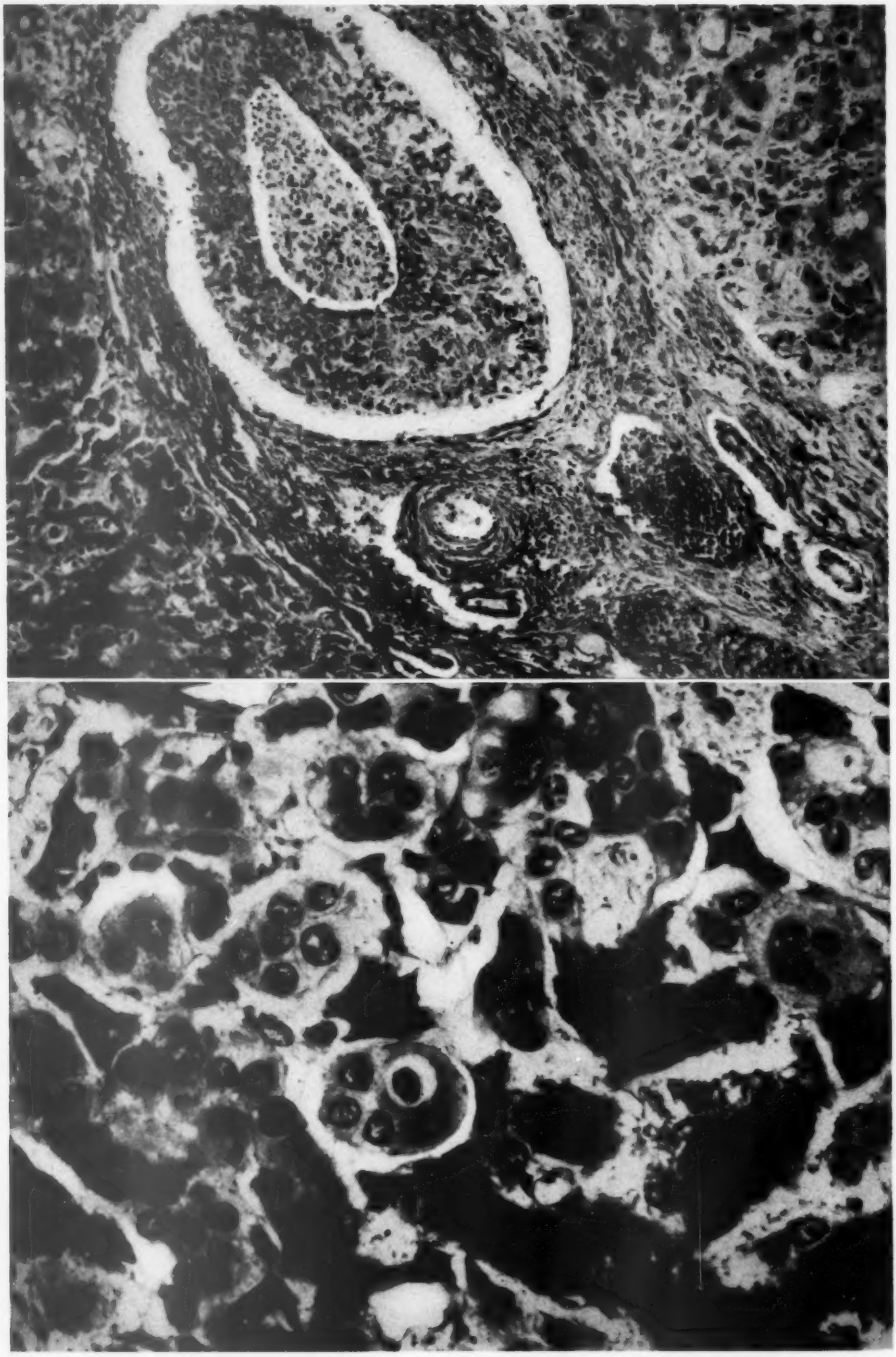

Warren and Drake Primary Hepatic Carcinoma in Hemochromatosis

PLATE 109

FIG. 12. Case 5. Lymphatic permeation at the periphery of a metastatic nodule in the lung. Despite anaplasia, polyhedral cells with prominent intercellular spaces suggest hepatic cords with bile canaliculi. Irregular cytoplasmic processes (also present in case 19) simulate those in normal stratified squamous epithelium. Phosphotungstic acid hematoxylin stain. × 405.

FIG. 13. Case 20. Pancreas with grade 4 pigmentation, atrophy, and fibrosis. Reduction in size of lobules and the amount of intervening fatty infiltration are striking. × 30.

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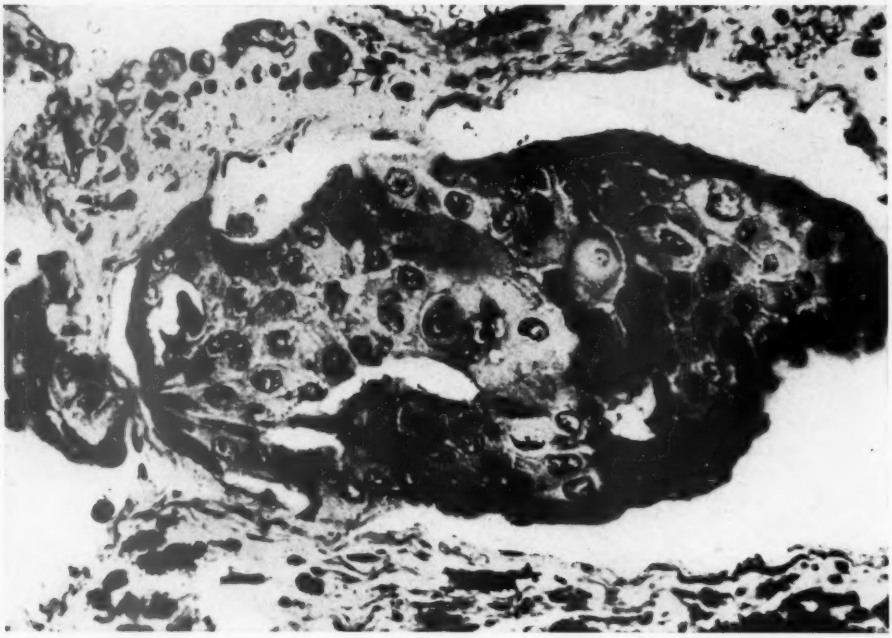

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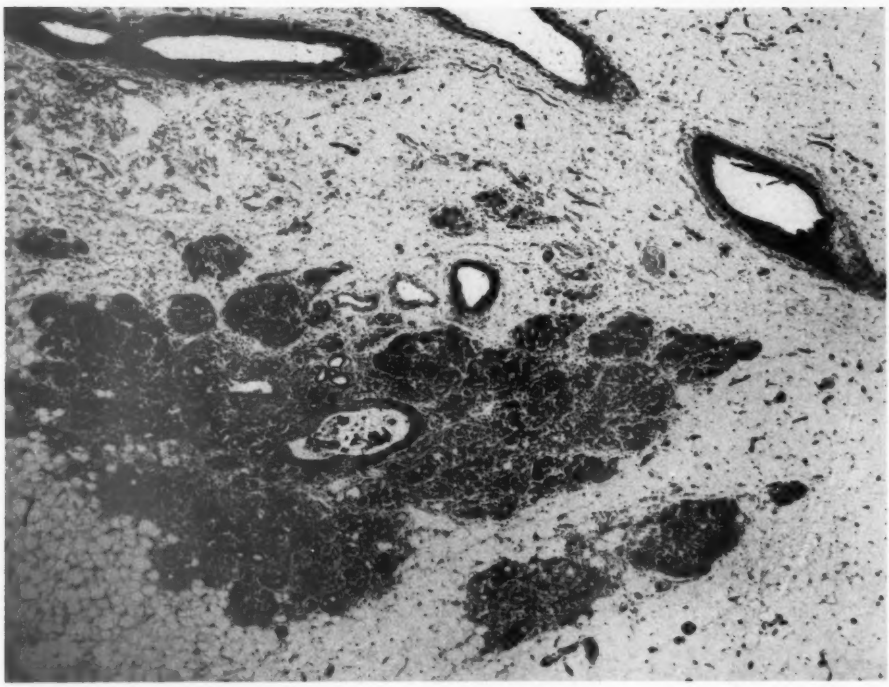


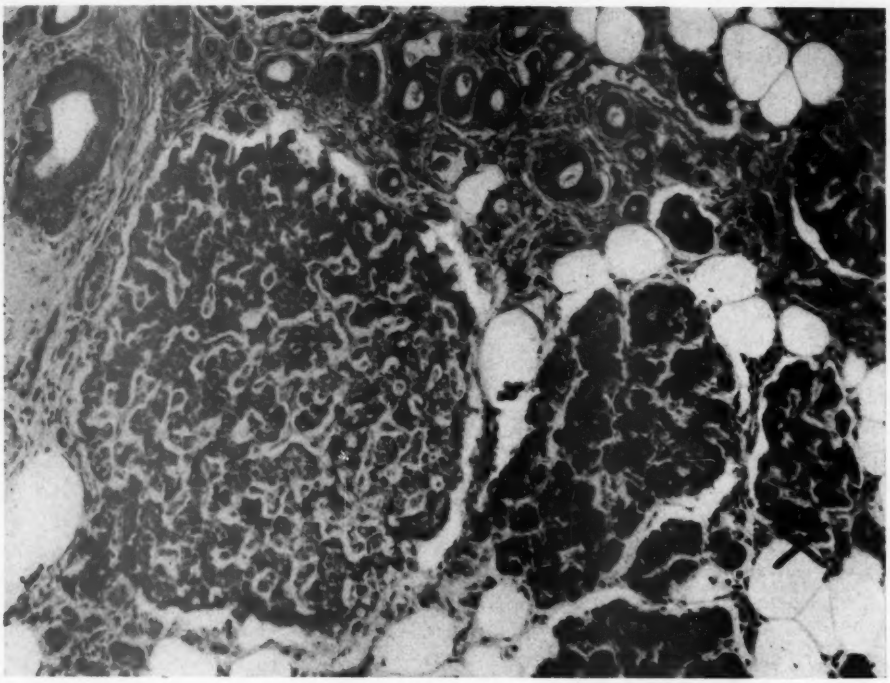
Warren and Drake Primary Hepatic Carcinoma in Hemochromatosis

PLATE 110

FIG. 14. Case 10. Hypertrophy of a pancreatic islet, uncommon in this series. Scantiness of pigmentation may be compared to that of an islet of average size at X. Greatest degree of pigmentation is present in acinar epithelium in contrast to islets and linings of ducts. × 125.

FIG. 15. Case 20. Discoid conglomerate masses of hemosiderin in the sinus of a lymph node. The central mass is being ingested by a foreign body giant cell. Lymph nodes of this case contained the only examples of this lesion throughout the series. Ferrocyanide stain, iron hematoxylin counterstain. × 395.

14



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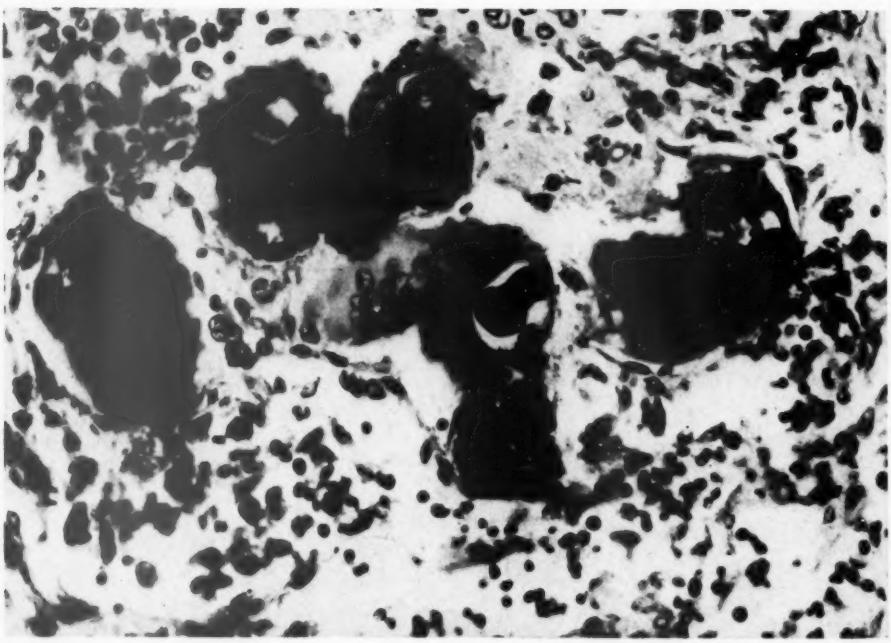

Warren and Drake Primary Hepatic Carcinoma in Hemochromatosis

PLATE III

FIG. 16. Case 7. Hemosiderin deposits in epithelium of choroid plexus of brain. Pigmentation here and at such sites as the glandular epithelium of the stomach (Fig. 17), adrenals, and thyroid gland, has been a criterion for differentiation of hemochromatosis from ordinary hemosiderosis. Ferrocyanide stain, iron hematoxylin counterstain. × 125.

FIG. 17. Case 7. Hemosiderin in both chief and parietal cells of gastric glands. The muscularis is at the right. Pigment was less abundant in the superficial layer of mucosa (not seen). There is a lack of pigment in the interstitial tissue. × 125.

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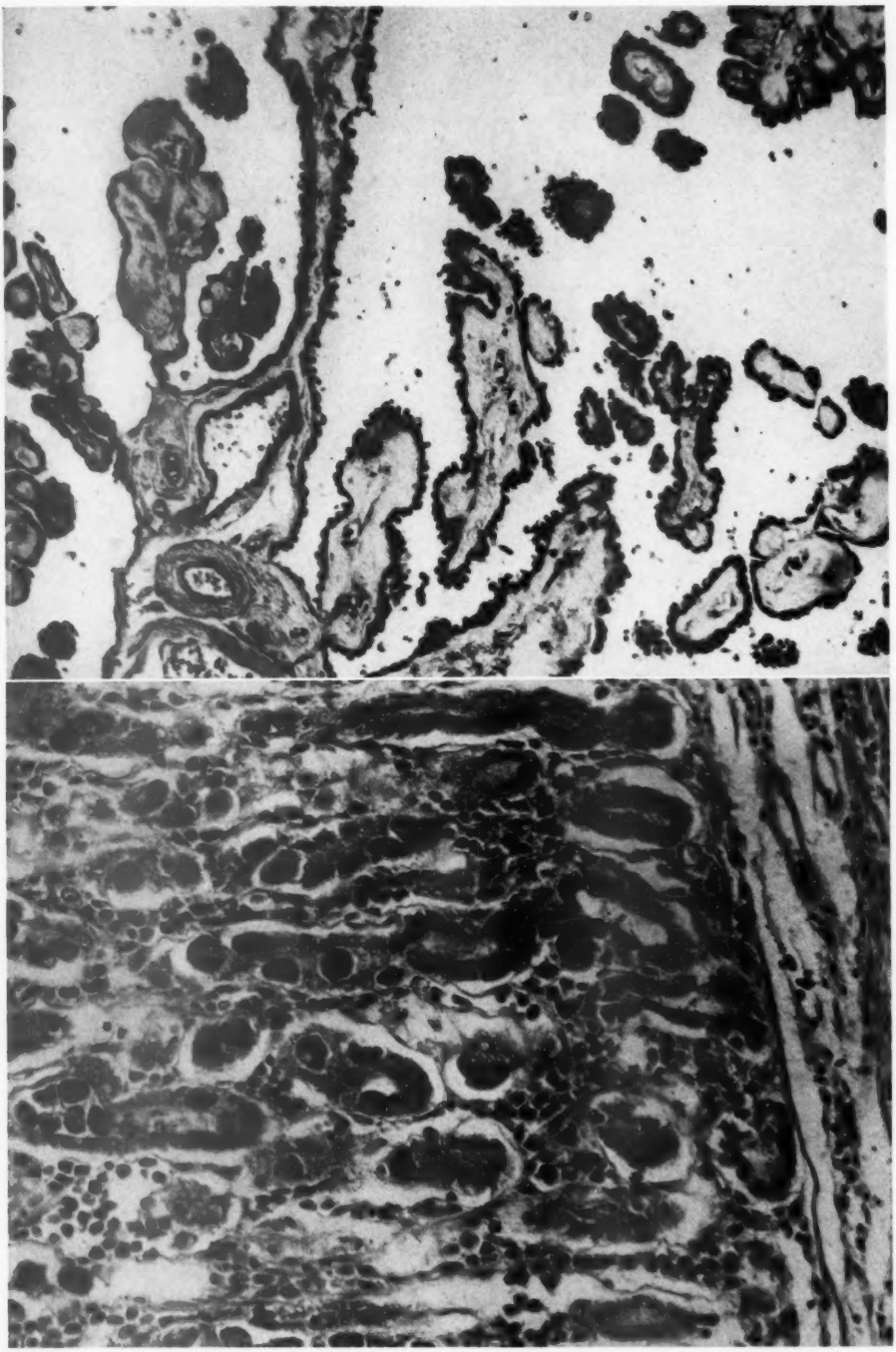

Warren and Drake Primary Hepatic Carcinoma in Hemochromatosis

HISTOCHEMICALLY DEMONSTRABLE GLYCOGEN IN THE HUMAN HEART

With Special Reference to Glycogen Storage Disease and Diabetes Mellitus *

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Histochemical study of glycogen in cardiac muscle previously has been hindered by lack of a sensitive, precise, and convenient technic. The periodic acid-leukofuchsin reaction, introduced independently by McManus [1] and Lillie,[2] has been shown to satisfy all of those requirements as a method for the coloration of glycogen. It is so sensitive that glycogen can often be demonstrated after aqueous fixation. With a given fixative, the periodic acid-leukofuchsin method consistently demonstrates more glycogen than either Best's carmine stain or the Bauer reaction.[2] Unfortunately, as with other methods, it is not specific for glycogen but colors a number of other substances in selective fashion. However, this source of possible confusion is easily circumvented by use of control sections which, prior to staining, have been treated with either diastase or saliva. The coloration of periodic acid-leukofuchsin positive material other than glycogen is apparently unaffected by such digestion procedures. In our experience, the diastase digestion procedure devised by Lillie and Greco [3] offers the advantages of more uniform action and ready adaptation to large numbers of slides compared with the salivary digestion procedures.

During the study of 2 cases of glycogen storage disease of the heart, reported elsewhere,[4] it was possible to compare the coloration of glycogen in sections by several staining methods. The superior performance of the periodic acid-leukofuchsin method led to a general inquiry of the occurrence of glycogen in infant and adult hearts. We hoped to establish standards that might permit use of this more sensitive method in the study of cardiac glycogen in diabetes mellitus, as well as in infants with glycogen storage disease. Warren [5] (1930) demonstrated marked glycogen infiltration of the myocardium in many untreated diabetic patients but concluded that little difference occurred between the diabetic patients treated with insulin and a control series of non-diabetic patients. The impressions were derived from study of Best's carmine stains on celloidin sections of tissue fixed in absolute alcohol. Yater,

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Osterberg, and Hefke [6] performed microchemical determinations of glycogen on both the muscle and the bundle of His in 21 human hearts, up to 9 hours post mortem. They concluded that the conduction system contained no more glycogen than the cardiac muscle. The concentrations of cardiac glycogen varied markedly from specimen to specimen without ascertainable reason. No diabetic cases were included in their series.

Materials and Methods

Thin blocks taken from the left ventricle were fixed at time of necropsy in either absolute alcohol or alcohol-formalin. Absolute alcohol was employed exclusively for fixation of the infant hearts. Alcohol-formalin (10 per cent formalin in 95 per cent alcohol) is an excellent fixing agent for glycogen and was used for all adults. As glycogen is known to be increased often in the margins of myocardial infarcts, such areas were carefully avoided in the selection of blocks.[5] Blocks of myocardium from 33 unselected infants, 17 known or suspected diabetic patients, and 63 non-diabetic patients have been studied. In a few instances successive blocks were removed from the same heart at increasing time intervals post mortem, in order to evaluate the influence of autolysis upon glycogen preservation.

After fixation, all blocks were dehydrated in absolute alcohol, cleared in xylol, and embedded in paraffin. Sections were cut at 10 μ without observing any unusual precautions. Sections were mounted from water on slides coated with glycerin-egg albumin and dried for 18 hours at 55° C. Omission of heat-drying frequently caused control sections to become detached during diastase digestion.

Periodic Acid-Leukofuchsin Reaction

The periodic acid-leukofuchsin reaction was performed essentially as prescribed by Lillie [7] except that potassium metaperiodate (KIO_4) was substituted for sodium periodate (Na_3IO_5), according to his most recent modification.[8]

1. Take sections through xylols and alcohols to distilled water.
2. Treat sections for 10 minutes with 0.8 per cent potassium metaperiodate dissolved in 0.3 per cent nitric acid.
3. Rinse in running tap water 5 minutes.
4. Transfer to Schiff's leukofuchsin reagent, prepared as directed by Lillie, for 15 minutes.*

* Dissolve 1 gm. basic fuchsin (C.I. no. 677) in 200 ml. boiling distilled water. Cool to 50° C. and add 10 ml. normal HCl and 2 gm. potassium metabisulfite ($K_2S_2O_5$). Allow to stand 24 hours. Then add 0.5 gm. neutral activated decolorizing charcoal or carbon. Shake 1 minute and filter. Filtrate should be light yellow to colorless and is ready for use.

5. Quickly rinse in three successive changes of M/20 sodium bisulfite ($NaHSO_3$), each for 90 seconds.
6. Wash in running tap water; counterstain at this point if desired.
7. Dehydrate, clear, and mount in neutral balsam. (Counterstains were not used in this study lest they interfere with grading of glycogen.)

Diastase Digestion Procedure for Control Sections

Prior to performance of the staining routine just described, control slides were incubated for 1 hour at 37° C. in 0.1 per cent U.S.P. IX malt diastase (Merck) freshly dissolved in Lillie's saline-phosphate pH 6.0 buffer.[9] The buffer contained 8.0 gm. sodium chloride, 0.282 gm. anhydrous disodium phosphate (Na_2HPO_4), and 1.974 gm. monosodium phosphate ($NaH_2PO_4 \cdot H_2O$) dissolved in 1000 ml. of freshly boiled distilled water. After digestion, slides were thoroughly rinsed in distilled water and stained by the periodic acid-leukofuchsin method. This control procedure was performed in every instance.

Slides were stained within a few days after cutting. However, no apparent loss of stainable glycogen has been observed after storage of either paraffin blocks or slides for many months. The effective life of the acidified periodate solution seemed to be determined largely by the number of slides treated, whether usage was occasional or intensive. Deterioration of its oxidizing action was easily detected by staining sections known to contain glycogen. Exhaustion was indicated by failure or decreased intensity of glycogen coloration. The reagent usually was discarded after exposure to about seventy-five slides. The leukofuchsin reagent was stored in the cold and considered satisfactory for use as long as it retained its original, practically colorless appearance. For reducing rinses, a stock molar sodium bisulfite solution was diluted for use and thereafter discarded. No evident fading of stained sections has been observed under ordinary conditions of use and storage.

Glycogen was identified in sections as deeply colored, purplish red granules that were absent in control sections. Within individual muscle fibers, glycogen usually was more concentrated on one side, an artifact described by others and known as polarity. Rather than proving troublesome, we found this distribution distinctive for glycogen and of some value in its recognition. With some practice it was possible to grade each section, in quasi-quantitative fashion, as containing 0 to 5 plus glycogen. The cytoplasm of muscle fibers was colorless or only faintly pink. Lipochrome pigment appeared in sharp contrast but retained its usual color, being unmodified by the technic. Besides gly-

cogen, connective tissue of stroma and vessels was consistently colored. Amyloid was easily detected in two instances by its characteristic dull pink coloration and waxy nature. Many mast cell granules were deeply colored and resistant to diastase digestion.

Results

For convenience, results from infant and adult hearts were considered separately.

Infants

The results are shown in Table I where cases are grouped according to histochemical glycogen content of the heart. It is evident that widely varying amounts of histochemically demonstrable glycogen were encountered. Cases graded as 1 plus generally contained only traces of glycogen and probably have no special significance. Excluding these, 19 of 33 infant hearts had definite and often considerable amounts of glycogen. Sections of heart from 2 cases of cardiac glycogen storage disease were studied under similar conditions and were assigned values of 4 plus glycogen by our standards. Thus, in 6 of 33 infants, quantities of glycogen comparable to those observed in glycogen storage disease of the heart were present. In 2 instances this quantity was exceeded (cases graded 5 plus). Furthermore, 3 of the 6 infants with abundant cardiac glycogen, graded as 4 or 5 plus, exhibited marked cardiac enlargement. The cause of this enlargement was unexplained in cases A50–183 and A50–31. There was coarctation of the aorta in the third case, A47–104.

The causes of death in our limited series were so varied that no correlation between disease and cardiac glycogen was deemed possible. An analysis of various other factors in relation to glycogen concentration in the heart is presented in summary form in Table II. For simplification, cases have been combined into three groups according to glycogen content: little or none (0 and 1 plus), intermediate (2 and 3 plus), and abundant (4 and 5 plus). The series was so limited that only a few generalizations seemed warranted. Hearts rich in glycogen were observed in a portion of all age groups studied, up to 8 months. Glycogen content did not appear significantly related to sex. While certainly of some importance, the interval between death and necropsy did not appear to affect the amount of demonstrable glycogen up to 12 hours and perhaps longer. In one instance abundant glycogen was shown 29 hours post mortem.

Adults

A series of 63 non-diabetic and 17 diabetic adults of various ages was studied for cardiac glycogen. For purposes of grading histochemically demonstrable glycogen, the two groups were studied together.

TABLE I

Glycogen in Infant Hearts

Cardiac glycogen	Necropsy no.	Sex, age	Hrs. p.m.	Weight of heart	Normal weight of heart	Major diagnoses
5 plus†	A49–273	F 3 mos.	29	30 gm.	23 gm.	Hydronephrosis; uremia
	A50–183*	F stillborn	3	50 gm.	17 gm.	Asphyxia neonatorum; periadrenal hemorrhage
4 plus	A47–104	M 10 days	5	Hypertrophied	19 gm.	Coarctation of aorta
	A49–47	M 2 mos.	8	25 gm.	23 gm.	Posterior urethral valve; uremia
	A50–31	M 6 mos.	3	58 gm.	31 gm.	Pneumonia; meningitis
	A50–89*	M 5 days	8	20 gm.	18 gm.	Amyoplasia congenita; bronchopneumonia
3 plus	A49–9	M 3 mos.	12	34 gm.	23 gm.	Sudden death; sepsis
	A49–57	M 2 mos.	7	19 gm.	23 gm.	Aortic atresia
	A49–64	F 2½ mos.	4	40 gm.	23 gm.	Panarteritis
	A49–100	M 5 mos.	?	Normal	29 gm.	Hepatitis
	A50–62*	M 4 hrs.	4	1.4 gm.		Prematurity
	A50–238*	F 8 mos.	13	50 gm.	37 gm.	Bronchopneumonia; atelectasis
2 plus	A47–23	M 5 mos.	6	66 gm.	29 gm.	Ostium primum
	A47–98	F 5½ mos.	3	44 gm.	29 gm.	Ependymoma
	A49–17	M 6 mos.	3	40 gm.	31 gm.	Bronchiolitis
	A49–126	F 6 mos.	10	38 gm.	31 gm.	Myelomeningocele; meningitis
	A49–186	F 8 mos.	3	37 gm.	37 gm.	Bronchopneumonia; poliomyelitis?
	A49–243	F 5 mos.	14	25 gm.	29 gm.	Myelomeningocele
	A50–151*	M ½ hr.	22	14 gm.	17 gm.	Prematurity; atelectasis
1 plus	A47–20	F 2½ mos.	6	49 gm.	23 gm.	Myocarditis
	A49–59	F 3 mos.	5	31 gm.	23 gm.	Myelomeningocele with hydrocephalus
	A49–104	F 5 mos.	2	Normal	29 gm.	Vascular ring; died postoperatively
	A49–133	F 7 mos.	4	88 gm.	34 gm.	Endocardial sclerosis; septal defect
	A49–224	F 2 mos.	9	Normal	23 gm.	Imperforate anus; peritonitis
0	A49–4	M 7 mos.	5	44 gm.	34 gm.	Acute laryngotracheobronchitis
	A49–92	M 2¼ mos.	?	Omitted	23 gm.	Rectosigmoid spasm, postoperative
	A49–133*	F 1½ mos.	17	32 gm.	21 gm.	Hemolytic *Staphylococcus aureus* abscesses in heart, lungs, and kidneys
	A49–169	M 4½ mos.	12	24 gm.	28 gm.	Amyotonia; pneumonia
	A49–198	F 8 mos.	6	35 gm.	37 gm.	Leukemia with hemorrhage
	A49–234	M 7 mos.	3	43 gm.	34 gm.	Sudden death; sepsis
	A49–271	F 3 mos.	4	22 gm.	23 gm.	Sudden death; sepsis
	A50–3*	M 3 days	5	17 gm.	17 gm.	Prematurity; atelectasis neonatorum
	A50–26	F 2 mos.	8	22 gm.	23 gm.	Hemangioma of neck

* All cases were necropsied at the Boston Children's Hospital except where indicated by an asterisk.

† *Schema for grading glycogen:* 5 plus: extreme and diffuse; 4 plus: abundant and generally diffuse; 3 plus: moderate and generally diffuse; 2 plus: slight or scattered foci; and 1 plus: trace amounts or isolated foci.

For comparison of the non-diabetic with the diabetic group, results have been recorded separately in Tables III and IV.

In contrast to the results of Warren,[5] we observed relatively little histochemically demonstrable glycogen in the hearts of non-diabetic patients. In 63 hearts examined, 3 had more than slight amounts of glycogen. None of the 3 cases showing more than 2 plus cardiac glycogen had any clinical or pathologic evidence of diabetes mellitus. In only one of them, A50–112, had there been administration of unusual amounts of carbohydrates. The other 2 patients with much cardiac glycogen were poorly nourished during their terminal illnesses. Ten non-diabetic patients showed slight amounts of cardiac glycogen; of these, 5 were cases of recent myocardial infarction. This was in contrast to the occurrence of only 4 instances of recent myocardial infarction in the remaining 50 cases containing only traces of cardiac glycogen or none.

TABLE II

Relation of Various Factors to Glycogen Content of Infant Hearts

	0 to 1 plus glycogen	2 to 3 plus glycogen	4 to 5 plus glycogen	Total number
Age				
Stillborn or premature	1	2	1	3 males 1 female
1 day to 7 weeks	1	0	2	1 male 2 females
2 to 4 months	7	3	2	5 males 7 females
5 to 8 months	5	8	1	6 males 8 females
Hours post mortem				
1–6	9	6	3	18
7–12	3	3	2	8
13–29	1	3	1	5
Not stated	0	1	1	2
Size of heart				
Normal	7	8	3	18
Enlarged*	6	5	3	14
Unknown	1	0	0	1
Sex				
Male	5	7	4	16
Female	9	6	2	17

* Hearts were considered enlarged when the excess in weight exceeded one-third normal.

TABLE III

Glycogen in Hearts of Sixty-Three Non-Diabetic Adults

Cardiac glycogen	Necropsy no.	Sex, age	Hrs. p.m.	Weight of heart and cardiac diagnoses	Other major diagnoses
5 plus	(None)				
4 plus	A50-31	M 90	4	425 gm.; minimal arteriosclerotic heart disease	*Bronchogenic carcinoma
	A50-112	F 60	12	390 gm.; normal	*Alcoholic cirrhosis
	A50-119	F 50	6	355 gm.; minimal coronary arteriosclerosis	*Myelomalacia; infarction of spinal cord
3 plus	(None)				
2 plus	A50-54	F 61	8	390 gm.; *recent as well as healed infarcts	Bronchopneumonia
	A50-74	M 56	6	400 gm.; hypertensive and arteriosclerotic heart disease with healed infarct	*Bronchogenic carcinoma
	A50-116	M 77	22	460 gm.; arteriosclerotic heart disease and *recent infarct due to coronary occlusion	Pulmonary edema; acute pyelonephritis
	A50-130	F 58	8	410 gm.; arteriosclerotic heart disease with *recent infarct	Chronic pyelonephritis
	A50-195	M 40	5	660 gm.; *rheumatic heart disease and slight arteriosclerosis	Pulmonary infarcts; bronchopneumonia
	A50-171	F 72	20	390 gm.; hypertensive and arteriosclerotic heart disease with *decompensation and recent infarct	Bronchopneumonia; benign nephrosclerosis
	A50-179	F 71	2	420 gm.; hypertensive and arteriosclerotic heart disease	*Cerebral hemorrhage
	A50-190	M 75	10	510 gm.; hypertensive heart disease and mural thrombus in right auricle	*Cerebral thrombosis; benign nephrosclerosis
	A50-191	F 53	7	390 gm.; acute myocarditis, minimal	*Carcinoma of cervix; acute pyelonephritis
	A50-214	F 65	8	375 gm.; arteriosclerotic heart disease and *recent infarct	None
1 plus	22 cases	Total of 50 cases consisting of 25 males and 25 females, ranging in age from 27 to 89 years, and dying from the variety of causes usually encountered in such material. Forty-two of the individuals were over 50 years old.			
zero	28 cases				

* Designates primary cause of death.

Of the hearts of 17 diabetic patients, 8 contained moderate to marked amounts of glycogen. A limited analysis of the diabetic care of these 8 revealed that 6 were receiving insulin, and that 3 appeared

TABLE IV

Glycogen in Hearts of Seventeen Diabetic Patients

Cardiac glycogen	Necropsy no.	Sex, age	Hrs. p.m.	Terminal control† and insulin dose	Duration and usual treatment of diabetes	Glycogen nephrosis	Weight of heart and cardiac diagnoses	Other major diagnoses
5 plus	A50–208	M 31	23	Poor; 20–10 u. PZI‡ q.d. (FBS 260-475)§	17 years; diet and insulin; frequent, severe reactions	Minimal	340 gm.; grossly normal	*Hypoglycemic encephalopathy
4 plus	A49–922	F 67	14	Good; 40 u. PZI q.d.	30 years; diet and insulin	None	325 gm.; arteriosclerotic heart disease; old infarct	*Myelomalacia
	A50–114	F 65	3	Good; only 2 u. PZI q.d.	7 years; insulin and deficient diet	None	330 gm.; healed infarct	*Carcinoma of stomach
	A50–72	F 78	7	Poor; 40 u. PZI q.d. (FBS 368)	11 years; diet and insulin	Moderate	500 gm.; *hypertensive and arteriosclerotic heart disease; aortic stenosis	*Severe heart failure
3 plus	A49–894	F 56	6	Poor; no insulin (FBS 344–211)	Diabetes not previously known	None	490 gm.; *recent myocardial infarction; hypertensive and arteriosclerotic heart disease	None
	A50–69	F 86	5	Poor; 15–20 u. RI‖ (4 + glycosuria)	Several years; diet and insulin, frequent reactions	Moderate	280 gm.; arteriosclerotic heart disease	*Bronchopneumonia (bilateral)
	A50–109	M 83	8	Good; 20 u. PZI q.d.	10 years; diet and insulin	None	350 gm.; arteriosclerotic heart disease; amyloidosis of heart (senile type)	*Bronchopneumonia (bilateral)
	A50–152	M 50	6	Fair; no insulin last 5 days, due to reactions	11 years; diet and insulin	Minimal	700 gm.; hypertensive heart disease with minimal decompensation	*Brain hemorrhages (multiple)

2 plus	A50–41	F 16	18	Died on arrival, diabetic coma not suspected	Diabetes not previously known	Marked	250 gm.; grossly normal	*Findings consistent with diabetic coma
	A50–170	F 69	23	Good; no insulin (FBS 119)	Several years; diet	None	590 gm.; *arteriosclerotic heart disease with decompensation	Infarcts of spleen, liver, and left kidney
	A50–200	F 72	12	Fair; no insulin last 4 days (FBS 325–172)	1 month; diet	None	700 gm.; *arteriosclerotic heart disease with decompensation	None
	A50–234	F 61	9	Poor; no insulin (FBS 191–465)	Several years; diet and, in past, insulin	Minimal	380 gm.; *acute myocardial infarction	*Encephalomalacia
1 plus	A50–146	M 66	20	Good; 10 u. PZI q.d.	12 years; erratic diet but took insulin	None	620 gm.; *arteriosclerotic heart disease with recent infarct	Bronchopneumonia
	A50–174	M 66	72	Improving; 50–25 u. RI q.d. (FBS 362–84)	Diabetes not previously known	None	420 gm.; *healing infarct with recent extension	None
0	A50–64	F 62	10	Poor; 70 u. RI last 9 hours (FBS 152–322)	Diabetes not previously known	None	540 gm.; *arteriosclerotic heart disease with decompensation; healed infarct	None
	A50–172	F 59	24	Poor; 30 u. PZI q.d. (FBS 230–304)	8 years; diet and insulin	None	480 gm.; minimal arteriosclerotic heart disease	*Encephalomalacia; bronchopneumonia
	A50–235	M 40	5	Poor; no insulin in last few days (severe reactions)	6 years; diet and insulin, frequent reaction	None	200 gm.; grossly normal	*Lung abscess and bronchopneumonia

* Designates major cause or causes of death.
† Terminal control refers to diabetic status during the last few days of life.
‡ Protamine zinc insulin.
§ Fasting blood sugar expressed in mg. %.
|| Regular insulin.

well controlled at the time of death. The group as a whole showed wide variation in cardiac glycogen compared with non-diabetic subjects. The differences did not appear clearly related to severity, duration, or treatment of diabetes. Glycogen nephrosis was present in only 4 of 8 cases with moderate to marked cardiac glycogen. Post-mortem interval appeared of some importance, as suggested by the fact that heart blocks from 5 of the 9 diabetic patients with 0 to 1 plus glycogen were fixed more than 22 hours after death. That post-mortem interval was of limited importance was indicated by finding 5 plus glycogen in A50–208, in spite of a delay of 23 hours and an illness accompanied by terminal hyperpyrexia.

Data from both diabetic and non-diabetic patients have been summarized and correlated with post-mortem time intervals in Table V. A general decline in incidence and amount of demonstrable glycogen

TABLE V

Relation of Post-Mortem Time to Glycogen Content of Heart in Eighty Adults

Amount of glycogen	Number of cases (diabetic cases in parentheses)				
	1–6 hrs.	7–12 hrs.	13–24 hrs.	24–72 hrs.	Total
3–5 plus	2 (4)	1 (2)	0 (2)	0 (0)	3 (8)
2 plus	3 (0)	5 (2)	2 (2)	0 (0)	10 (4)
0–1 plus	15 (1)	19 (1)	13 (2)	3 (1)	50 (5)
Total	20 (5)	25 (5)	15 (6)	3 (1)	63 (17)

was observed with increasing time for both groups. That the post-mortem disappearance of glycogen, at least in diabetic patients, is related to the initial concentrations of glycogen was shown by both these results and several autolysis experiments. Consecutive blocks were fixed at varying intervals from 3 glycogen-rich diabetic hearts stored at 5° C. The results of these glycogen studies were: A50–72, initially 4 plus, showed 2 plus glycogen after 22 additional hours; A50–114, initially 4 plus, was 3 plus after 18 additional hours; A50–208, initially 5 plus, was 4 plus after 48 additional hours, and 3 plus after an additional 120 hours. The original time post mortem in each of these cases is recorded in Table IV.

DISCUSSION

In Van Creveld's [10] extensive survey of glycogen storage disease, he reviewed a number of previously reported cases constituting a form of the disease primarily affecting the heart and apparently distinct from the more common hepatic form, or von Gierke's disease. Glycogen storage disease of the heart, known variously as cardiac glycogenosis

and cardiomegalia glycogenica, is characterized by considerable cardiac enlargement without known functional cause and the presence of glycogen diffusely distributed throughout the myocardium in concentrations greatly exceeding those believed to occur normally. The pathologic amounts of glycogen usually have been shown by Best's carmine stain, occasionally by chemical determinations. Demonstration of abundant glycogen in sections, despite unfavorable fixation and time post mortem, has been considered evidence of abnormal resistance of the glycogen to autolysis. A limited number of chemical determinations have shown little or no change in glycogen concentration after storage of hearts.

Our results show that small hearts as well as enlarged hearts, whether due to demonstrable cause or idiopathic, may often contain abundant amounts of histochemically demonstrable glycogen. Whatever the mechanism controlling concentrations of glycogen in the heart may be, it seems to have no definite relation to factors producing cardiac enlargement. The frequency with which large amounts of histochemically demonstrable glycogen have been found in infant hearts not only complicates the diagnosis of glycogen storage disease but emphasizes the lack of evidence that glycogen is causally related to cardiac enlargement. The mere demonstration of abundant glycogen in sections from a heart showing idiopathic hypertrophy would seem insufficient justification for placing the case in an entirely different nosologic category, such as glycogen storage disease of the heart. It is not our intention to question the existence of glycogen storage disease of the heart but to stress that glycogen infiltration of the heart is in no way specific for this disease.

One feature described for glycogen storage disease of the heart needs further amplification, namely, that the glycogen is resistant to autolysis.[10] Studies of this kind, together with quantitative glycogen determinations in a statistically adequate series of control infants, might place this disease on a firmer diagnostic basis. With regard to resistance of glycogen to autolysis, it may be noted that one of our infants showed 5 plus cardiac glycogen after 29 hours post mortem. The apparently slower post-mortem disappearance of glycogen in hearts of infants, compared to adults, is probably related to the more rapid cooling of the small infant body and, possibly, to the lower diastase concentrations reported to occur in blood of infants.[11]

Warren's [5] finding of abundant glycogen in the hearts of many diabetic patients has been confirmed by the periodic acid-leukofuchsin technic. In addition, this finding seems more distinctive for diabetes and less specifically related to lack of insulin therapy than originally

proposed. Abundant amounts of cardiac glycogen are uncommon in non-diabetic subjects, especially in cases more than 6 hours post mortem. On the other hand, large amounts of glycogen were found in 4 of 5 diabetic hearts examined within 6 hours and in about one-third of the cases fixed many hours post mortem. Extensive glycogen infiltration of the heart seems confirmatory, if non-specific, evidence of diabetes mellitus. The significance and mechanism of moderate to marked amounts of cardiac glycogen in occasional non-diabetic and many diabetic patients are obscure at present. There is no evidence that its presence in any way alters the function or size of the heart. Results of histochemical liver glycogen determinations performed on most of the cases presented, reveal no evident correlation with cardiac glycogen.

Summary

The periodic acid-leukofuchsin reaction and diastase digestion of duplicate slides as controls are useful procedures in the histochemical study of cardiac glycogen. These methods were applied to the study of glycogen in the myocardium in 33 infants, 63 non-diabetic adults, and 17 diabetic patients. The amounts of histochemically demonstrable glycogen were found to vary widely in hearts of infants, often approaching and sometimes exceeding amounts seen in glycogen storage disease of the heart.

In hearts from non-diabetic adults only slight amounts of glycogen were seen with frequency. In a few instances, however, abundant amounts were observed. In hearts of diabetic patients, on the other hand, glycogen was frequently present in moderate and often in large quantities. Increased amounts of histochemically demonstrable glycogen in diabetes mellitus were not clearly related to severity, duration, control, or nature of treatment.

The results indicate that extensive glycogen infiltration of the heart is common in infancy and in diabetes mellitus. The presence of massive amounts of histochemically demonstrable glycogen in the myocardium is neither infrequent nor specific for glycogen storage disease of the heart.

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[*Illustrations follow*]

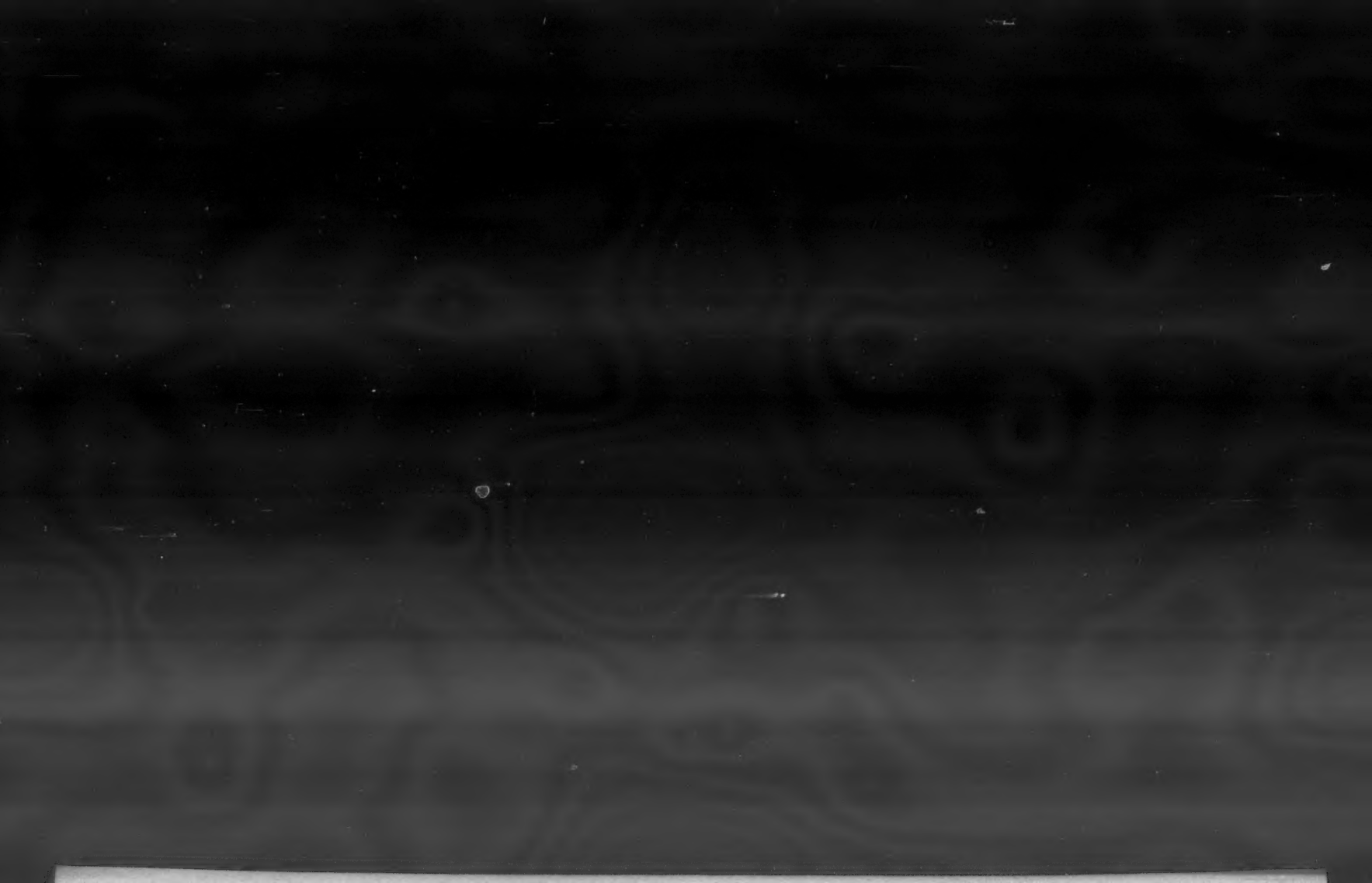
DESCRIPTION OF PLATE

PLATE 112

Both illustrations were prepared from sections stained by the periodic acid-leuko-fuchsin technic without nuclear counterstain.

FIG. 1. Myocardium of a diabetic patient showing diffusely distributed glycogen, grade 4. × 300.

FIG. 2. Control of case shown in Figure 1, adjacent serial section, diastase digested. Glycogen has been removed completely. The dark, granular material remaining is all lipochrome pigment. × 300.



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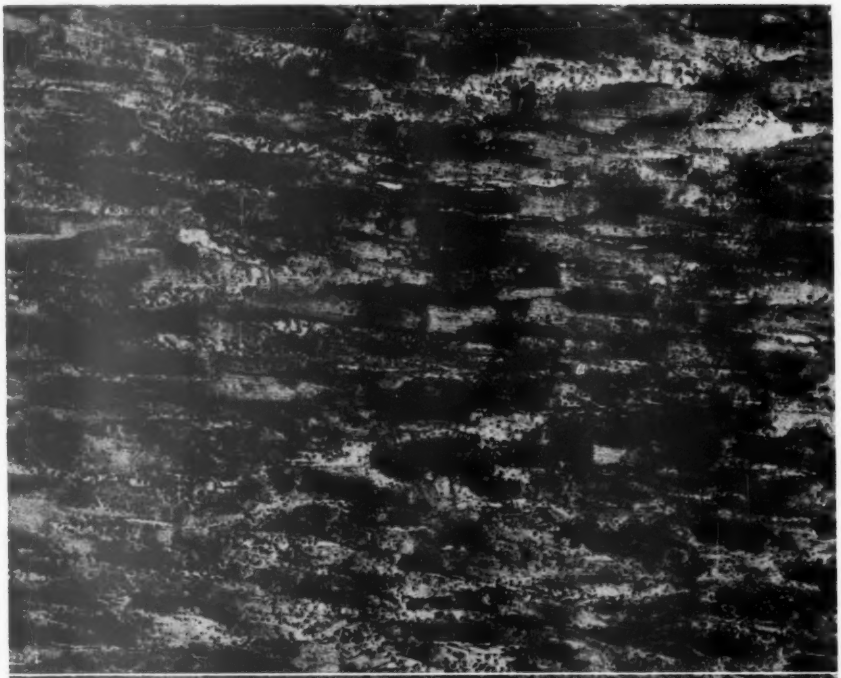

2

Mowry and Bangle Glycogen in the Human Heart

THE PRIMARY RETICULO-ENDOTHELIAL GRANULOMAS

With Report of an Atypical Case of Letterer-Siwe's Disease *

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The widened concept of non-lipid reticulo-endotheliosis as a systemic disease with three general expressions has received recognition in the American literature within recent years. Separation of Hand-Schüller-Christian disease from the xanthomatoses was paralleled by the identification of Letterer-Siwe's disease and eosinophilic granuloma of bone. Subsequently, all three were grouped as clinical variants of a basic disorder of the reticulo-endothelial system, and they are so considered by many investigators today.

It is the purpose of this report to describe a case which, although not clearly corresponding to any one of three variants, is believed to represent a non-lipid reticulo-endothelial granulomatous disease. A critical review of the pertinent literature is included also.

Review of Literature

During the past 2 decades there have appeared many reviews treating the entire problem of reticulo-endotheliosis. It should suffice to trace chronologically the significant stages to the present by which Christian-Schüller's, Letterer-Siwe's, and eosinophilic granuloma of bone syndromes have come to be grouped, more or less, as related entities.

The discovery of the reticulo-endothelial system during the decades from 1880 to 1930, and the fundamental work of Aschoff, Kiyono, and others, gave impetus to the initial studies of a previously unrecognized disease characterized by proliferative changes in this system. In the light of subsequent knowledge, many of the early cases came to be classified as Christian-Schüller's disease. Cases reported by Hand [1] in 1893, Kay [2] in 1906, Schüller [3] in 1915, and Christian [4] in 1919, in particular, led to the characterization of the disease as a triad comprising exophthalmos, diabetes insipidus, and defects in the membranous bones. The earliest known case of Christian-Schüller's disease, according to Fraser,[5] was described by Smith [6] in 1865. Weidman and Freeman,[7] under the title of xanthoma tuberosum, reported a case of Christian-Schüller's disease which presented cutaneous, visceral, and

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central nervous system involvement. They suggested that the foamy lipid-containing cell, which had been previously observed, was the basic cell of the lesion, and, further, they believed that the lipid resulted from cholesterol infiltration. Epstein [8] was the first to suggest that the standard triad was not always present. Rowland,[9] in 1928, analyzed 14 cases with the triad picture, and gave a complete description of the histopathologic changes. He placed emphasis on the foam cells as indicating a generalized lipid storage disease. In the following year he stated [10] that these characteristic cells develop secondary to hyperlipemia. Chester [11] suggested that Christian-Schüller's disease was a type of lipid granuloma.

Henschen,[12] in 1931, on the basis of a review of 57 cases, pointed out the actual variability of age, symptoms, lesions, and other aspects. In contrast to the then current concept that Christian-Schüller's disease could be diagnosed only when the classical triad of signs was present, he defined eight general types, several of which did not exhibit the triad. According to Mallory,[13] Bürger had previously found 22 of 48 cases of Christian-Schüller's disease to be atypical. Henschen's thesis subsequently was extended through intense study of the nature, distribution, and chemical characteristics of Christian-Schüller's disease, as reviews and reports by many investigators [14-19] were added to the literature.

Further pertinent studies [20-26] initiated the debate over the validity of continuing to identify Christian-Schüller's disease with the primary lipid storage diseases. Though Fraser [5] held that high levels of cholesterol in body fluids and blood led to lipid absorption by reticulo-endothelial cells, and Sosman,[27] Teperson,[28] and Rowland,[29] among others, adhered to the original concept of lipid storage abnormality, lesions without lipid often were noted but never explained. Versiani, Figueiró, and Junqueira,[30] cited Snapper [30a] as hypothesizing that reticulo-endothelial proliferation was the primary event in Christian-Schüller's disease, while Ceelen [31] considered cholesterol deposition as secondary. Thannhauser and Magendantz,[32] though believing Christian-Schüller's disease to be a primary xanthomatosis with high tissue levels of lipid, nevertheless noted the absence of hypercholesterolemia.

At about this time, a related disease complex, subsequently called Letterer-Siwe's disease, was gaining wide recognition. Letterer,[33] in 1924, had given the first detailed description of a case which demonstrated the existence of a non-lipid form of reticulo-endothelial hyperplasia. Three similar cases were reported in 1926 by Wollstein and McLean,[34] Krahn,[35] and Akiba,[36] and 3 more in 1931 by Guizetti,[37] Podvinec and Terplan,[38] and Goldzieher and Hornick.[39] The last-

named authors, under a related form of so-called reticulosis, cited 22 cases in the literature, from 1903, when Borissowa [40] reported his case, to 1931. Similarly, Dameshek,[41] writing on aleukemic reticulosis, which he suspected to be a form of Hodgkin's disease, accepted 13 previously reported cases. A well defined entity of eight clinical and pathologic requirements was set up by Siwe [42] in 1933, from 3 prior cases and one of his own. Galeotti Flori and Parenti,[43] in 1937, were among the first to suggest that Christian-Schüller's and Letterer-Siwe's diseases were essentially similar.

From time to time various classifications reflected the difficulties of segregating the increasing number of reticulo-endothelial disorders coming into view. All of these were classified by Epstein [8] as histiocytomatoses and by Uehlinger [44] as reticuloses, but each of these workers included Christian-Schüller's, Niemann-Pick's, and Gaucher's syndromes under the prime category of lipid storage disease. Van Creveld and Ter Poorten [45] delimited three categories of reticulo-endothelial disorder: the first, a response to typhoid and tuberculous infections; the second, the lipidoses; and the third, the reticulo-endothelioses. Abt and Denenholz [46] widened this classification to six forms, including infections, lipidoses, Letterer-Siwe's disease, Hodgkin's disease, leukemias, and neoplasms. Rowland [29] adhered to the above pattern, but he made his division on the basis of acute and chronic types, including the xanthomatoses as examples of Christian-Schüller's disease. Sacks,[25] while defining reticulo-endotheliosis as a systemic proliferation intermediate between inflammation and malignancy, considered monocytic leukemia, retothelial sarcoma, and reticulo-endotheliosis as the prime forms.

Resolution of existing knowledge toward a common path was undertaken by Wallgren [47] in 1940. After careful study of the literature and of 23 cases of both Christian-Schüller's and Letterer-Siwe's disease, and combining the essential thinking of his predecessors, he concluded that infection is not causal, that Christian-Schüller's disease is not a xanthoma, that cholesterol deposits are secondary, and that Christian-Schüller's and Letterer-Siwe's diseases are the same disease with different histologic phases due to the chronic and acute nature of their respective courses.

At this stage interest was focused on a third form of reticulo-endothelial disorder which received foremost attention during the following 8-year period. Five authors [5,48-51] had seen such cases before Otani and Ehrlich [52] and Lichtenstein and Jaffe,[53] in 1940, independently recognized and comprehensively analyzed the process. This consisted of a solitary granulomatous lesion of bone, the predominant histologic cell

type being the eosinophil. These authors considered the disease to be a disorder of the reticulo-endothelial system, separate and distinct from Christian-Schüller's and Letterer-Siwe's diseases. Five additional cases were reported [54-57] in 1940 and 1941 illustrating multiple as well as solitary lesions in long and membranous bones. This disease has been named eosinophilic granuloma of bone.

Farber,[58] in 1941, presented 5 new cases of eosinophilic granuloma of bone, and suggested that Christian-Schüller's disease, Letterer-Siwe's disease, and eosinophilic granuloma of bone were variants of the same basic disease process. With Green,[59] he extended his observations and maintained his earlier opinion. Gross and Jacox [60] supported him and questioned whether a sharp distinction could justifiably be made between the lipid and non-lipid reticulo-endothelioses.

Since then, controversy has existed concerning the true relation of the three conditions. Impressed by intermediate stages in his own and in other cases, Mallory [13] found the evidence strongly suggestive that the three conditions were variants of one non-lipid-storage disease. He differentiated them on the basis of patients' ages, distribution of lesions, and histopathologic findings. Mallory's conclusion was partially upheld by Jaffe and Lichtenstein,[61,62] who, in accord with Green and Farber,[59] believed that there was an exact clinical distinction between the three expressions of the same fundamental pathologic disorder. Yet they disagreed with the general opinions of Green and Farber, and of Engelbreth-Holm, Teilum, and Christensen, [63] that the natural morphologic history of eosinophilic granuloma of bone was necessarily a progression from proliferation to granulomatosis to xanthomatosis to fibrous healing. Thannhauser,[64,65] however, accepted the four-stage cycle. He viewed eosinophilic granuloma of bone as the early monosymptomatic stage of the histologic Christian-Schüller's lesion, and persisted in ranking Christian-Schüller's syndrome as a xanthomatous disease. Weaver and Carter [66] thought the evidence to be overwhelming that eosinophilic granuloma of bone is a variant of Christian-Schüller's disease, but others [67-72] preferred to look upon all three syndromes as the same reticulo-endothelial disease.

More recent opinions have continued to vary. Laymon and Sevenants [73] found a lack of concrete evidence that Letterer-Siwe's and Christian-Schüller's syndromes are separate diseases. Some observers [72-74] held to the opinion that Christian-Schüller's disease is not a xanthoma. Schuknecht and Perlman [74] admitted the close relationship between Christian-Schüller's disease, on the one hand, and Letterer-Siwe's disease and eosinophilic granuloma of bone on the other. They stated that the lesions of eosinophilic granuloma of bone are really mani-

fested in four possible phases, but suggested that this does not imply that the lesions develop or heal by passing through all four states. Schafer [75] noted the close relationship between Letterer-Siwe's and Christian-Schüller's diseases, but believed that the relation of both of these to eosinophilic granuloma of bone is less clear. She was of the opinion that two or more conditions must be similar in course and clinical features as well as in anatomical structure in order to be accepted as variations of the same disease. She provided evidence that foam cells do not always represent chronicity of lesions as was generally thought. Havard, Rather, and Faber [76] remarked that histologic interrelationships do not necessarily imply identity of etiologic agent.

Arrival at these attitudes was in large measure due to the accumulation of many cases which either were not typical of a prescribed syndrome, or exhibited features of more than one syndrome, or of some other condition. For example, the diagnosis of Merritt and Paige's [21] case seems to have changed, in the opinions of others, from Christian-Schüller's disease to Letterer-Siwe's disease. Between 1933 and 1942 there were reported 16 cases,[18,23,26,43,60,77-86] presenting features of Letterer-Siwe's disease, and also of Christian-Schüller's disease, combined with simultaneous lipid and non-lipid lesions in widespread organic distribution. Such variations from the norm, and grades of transition from one to another type, even histologically, were alluded to by Wallgren [47] and by Mallory.[13] Also, a number of cases [87-91] of supposed reticulo-endothelial disease have never been universally accepted as such.

In an analysis of 84 cases of Christian-Schüller's disease, Gross and Jacox [60] found 9 definitely without lipid cells and 29 with blood eosinophilia. They reported cases of eosinophilic granuloma of bone that later were suspected by Greenberg and Schein [67] to be Christian-Schüller's disease. Jaffe and Lichtenstein [61] pointed out known and probable similarities in osseous lesions between Christian-Schüller's disease and eosinophilic granuloma of bone and between Letterer-Siwe's disease and Christian-Schüller's disease in various age groups. One of their 12 cases of eosinophilic granuloma of bone was labeled Letterer-Siwe's disease by Schafer.[75] Multiple eosinophilic granuloma of bone was likened to, or confused with, Christian-Schüller's or Letterer-Siwe's disease at times. Laymon and Sevenants [73] re-emphasized the extreme variability of Christian-Schüller's disease and cautioned that the roentgenologic appearance of all three syndromes cannot be differentiated. Further discrepancies and parallels were supplied in later reviews.[72,74,75,92]

As for eosinophilic granuloma of bone, it has been found with extra-

skeletal extension. After Engelbreth-Holm *et al.*[63] stated that the name eosinophilic granuloma applied to cases with lesions in other organs besides bone, lesions of eosinophilic granuloma were noted in the skin,[69,73,93,94] lungs,[70,71,95,96] and ears.[74] The total incidence, moreover, is quite high, for whereas there were only 24 reported cases of Letterer-Siwe's disease up to 1948,[75] there were 62 of eosinophilic granuloma of bone by 1946,[97] and there have been at least a dozen since.[66,69-72,74,96,98]

Cutaneous eosinophilic granuloma has been the subject of some confusion. A distinctly new dermatologic concept was presented in 1947 by Weidman,[99] by Lewis and Cormia,[94] and by Lever [92] under the name of eosinophilic granuloma of the skin. These authors regarded this lesion as a tissue response produced by a wide range of systemic diseases, in which the skin showed characteristic perivascular eosinophilic infiltrations and arteriolar endarteritis. This was obviously not the same process as the skin involvement which occurs in eosinophilic granuloma of bone, and the authors did not establish a true relation between the skin lesions of their cases and those found in eosinophilic granuloma of bone. Laymon and Sevenants [73] later collected from the literature what they believed were the true prior examples of eosinophilic granuloma of bone with skin involvement, and gave a detailed microscopic description which clarified the differences between the two types of skin lesions.

From the above survey, it is apparent that the classification of these reticulo-endothelial aberrations is far from settled. When individual cases cannot be distinguished with necropsy material at hand; when histologic changes, still incompletely studied and identified, are so diversified and yet so alike; and when the clinical courses stray from the pattern, it is not to be wondered that questions should arise, and, as Wallgren [47] observed, that opinions should differ.

Report of Case

In June, 1946, at 30 years of age, the patient, a male clerk, first came under hospital care at another institution, complaining of severe thirst, urinary frequency and polyuria, and loss of appetite, strength, and weight.

Seven years earlier, in 1939, he had received surgical care for an infection of a foot, the exact nature of which is not known. At that time, and for several years thereafter, he was employed as a metal grinder. In 1942 he was first troubled by a chronic discharge from the right ear, and by a disorder of the scalp diagnosed as seborrheic dermatitis. He served with the Navy between 1943 and 1945, spending 6 months of that period in the South Pacific area. His only illness during that time was a fever of unknown etiology lasting 2 days.

His present illness began in April, 1946, with insidious onset of increasing thirst, polyuria, and nocturia. Within 3 weeks he experienced loss of appetite and strength. At the time of his first hospital admission in June, 1946, he had lost 7 lbs. in weight,

and was consuming 6 l. of fluids daily. On this admission his heart and lungs were normal, both on physical and on roentgenologic examination (Fig. 1). At this time the specific gravity of his urine never exceeded 1.002. A phenolsulfonphthalein test revealed 75 per cent of the dye excreted in 2 hours. Blood counts were normal. He was placed on pitressin therapy, which successfully controlled his fluid output during the ensuing year. In July, 1947, he was examined at a large clinic, where the first skull plates were secured. These were found to be negative. His medication was adjusted, and he was discharged.

The first of numerous and repeated episodes of respiratory symptoms appeared 2 months later in the form of a mild head cold with sore throat, nasal discharge, and slight cough. The cough persisted, and for a brief period was productive of mucoid sputum. This was followed by sudden, severe, lancinating pains in the right lower chest, lasting 5 days. A month later he experienced similar pains in the left chest. By November, 1947, distressing dyspnea and malaise were becoming evident.

Because of persistent chest pain, dyspnea, and cough, he was admitted to the New Britain General Hospital for the first time in December, 1947. Bronchial breathing and coarse râles were noted at the base of the left lung. Chest films disclosed a cyst-like fibrotic process in both lower lobes. The total white blood cell count was 7,300 per cmm. with a differential count of 83 per cent polymorphonuclear neutrophils. The sedimentation rate was 35 mm. in 1 hour (Westergren). The electrocardiographic tracing showed no deviation from the normal. Sputum and gastric washings failed to reveal acid-fast bacilli.

The patient was transferred to a Veterans Administration Hospital during his second hospital week. Cough was then minimal, but the temperature mounted several times to 103° F. It was discovered that there had been a weight loss of 17 lbs. in the preceding 8 months. Scattered over the scalp were a number of hemorrhagic, ulcerated lesions measuring from 1 to 3 mm. in diameter. At the lung bases were dullness to percussion, absent to diminished breath sounds, and decreased fremitus. The vital capacity was 1.2 l. (normal, 4.3 l.), and chest films showed interstitial fibrosis in both lower lobes and the right middle lobe. Roentgenograms of the skull were negative. The white blood cell count was now 11,800 per cmm., 72 per cent of which were neutrophils. The sedimentation rate was 28 mm. in 1 hour. The cold agglutination test was negative. Three gastric washings were negative for acid-fast bacilli. Two hundred cc. of bloody fluid, removed from the left chest, contained numerous neutrophils, but cultures for bacteria and fungi were negative. On bronchoscopy there was reddening of the bronchial mucosa, but aspirated secretions, when cultured on diverse media and studied by Gram, Papanicolaou, and acid-fast stains, were negative. Thoracotomy was refused by the patient. Before discharge on December 2, 1947, an unsuccessful diagnostic attempt was made to discontinue pitressin.

The patient returned to his work as a clerk and was troubled only by mild cough until February, 1948, when he was readmitted for a brief period to the original Veterans Administration Hospital because of severe dyspnea. His vital capacity on this admission was unimproved. A right bronchogram showed good filling and some displacement of the middle and lower lobe bronchi, and no bronchiectasis. The alveoli of only the upper lobe were entered by the dye (Fig. 2).

Continuing moderate cough and dyspnea with a sudden attack of violent coughing and right chest pain caused him to enter a third hospital in November, 1948. At this time he appeared chronically ill. He had tachycardia, and his temperature was 99° to 101° F. On the forehead and chest were several small, scarred and pustular lesions. The right tympanic membrane was retracted and covered by purulent exudate. From this exudate *Bacillus proteus* and *Escherichia coli* were cultured. There were moist râles at each lung base and decreased breath sounds at the right base. Chest films were interpreted as indicating idiopathic pulmonary fibrosis with diffuse emphysematous bullae and partial right pneumothorax. Roentgenograms of the skull

and bones of the hands were negative. Laboratory studies showed a white blood cell count of 10,500 per cmm., with 84 per cent neutrophils. The total serum protein was 7.8 gm. per 100 cc. with a reversed albumin-globulin ratio of 3.8/4.0. Complement-fixation and precipitin tests for coccidioidomycosis and histoplasmosis were negative. Tissue taken for biopsy from one of the skin lesions showed many mononuclear phagocytes and a few round cells not characteristic of any specific disease. The pathologic diagnosis was chronic dermatitis. The patient was treated symptomatically. There was some improvement, with 50 per cent absorption of the pneumothorax in 2 weeks.

The seventh and last hospital admission occurred on December 31, 1948, when he entered the New Britain General Hospital. At this time the patient was in extremis, with a temperature of 102.8° F., pulse of 120, and respiratory rate of 64. There were coarse inspiratory râles throughout both lung fields, but the heart sounds were of good quality. The hemoglobin determination showed 11 gm. per 100 cc., and the white blood cell count was 12,500 per cmm. There were a few red blood cells and pus cells in the urine. Fever continued, respirations grew more labored, coma intervened, and he expired on January 2, 1949.

POST-MORTEM EXAMINATION

A necropsy was performed 4 hours after death. There was slight cyanosis of the hands and face. On the chest and scalp were several scattered, superficial, papular and ulcerated lesions having a diameter varying from 1 to 3 mm. The lesions on the scalp were confined within the hairline. They were scaly and a few were hemorrhagic. Those on the chest were scarred. There were dense and delicate, shaggy adhesions uniting the pleural surfaces bilaterally. The pleura was dull, markedly thickened, and speckled gray-white with scattered hemorrhagic mottling, particularly over the lower lobes of the lungs. There was no free fluid in the peritoneal cavity, and the peritoneal surface was glistening.

The right lung weighed 655 gm. and the left lung, 660 gm. External palpation revealed many scattered, nodular zones. The cut surface of both lower lobes, the right middle lobe, and the lower portions of the upper lobes presented many small and large, irregular, ramifying and intercommunicating cystic spaces and canals (Figs. 3 and 4). These varied in diameter from a few millimeters to several centimeters. They were lined by a shiny or dull, grayish white surface, and contained either air or a light muddy-brown fluid. External to the cysts the tissue often was dense and fibrous. Many of the cysts seemed to arise from nodular lesions. Bridging the cysts were intact bronchioles and blood vessels. Attempts to demonstrate a communication between the bronchioles and the cystic spaces, by injecting air or water under pressure into the bronchioles, were unsuccessful. Most of the upper lobe parenchyma was firm and consolidated, the cysts having formed in proximity to the hilum. Small emphysematous blebs were noted on all pleural surfaces, and were most numerous at the apices. The only

parenchymal tissue spared by the disease was in the superior margins of the upper lobes. The lumina of the bronchi contained abundant foamy, serous fluid.

The heart weighed 315 gm. The musculature was flabby. Except for right-sided hypertrophy and dilatation, there were no gross changes. The spleen was congested and had a taut, slightly thickened capsule. It weighed 250 gm. The malpighian corpuscles were not enlarged. The liver weighed 1445 gm. It was slightly congested. The kidneys also were congested, and their pelvic mucosa slightly injected. Only the hilar and adjacent mediastinal lymph nodes were enlarged. They were soft and discrete. The cut surface showed some gray streaking and anthracotic pigment. The brain weighed 1485 gm. Slight edema was present. The anterior lobe of the pituitary body was moderately enlarged.

Microscopic Examination

Lungs. The principal change in the lungs was a widespread, infiltrative, cellular proliferation arising from peribronchial and interstitial tissues (Figs. 5 to 7). The cells extended irregularly in all directions into the surrounding lung parenchyma, completely replacing the latter in large areas of the lower lobes. The process varied from dense, compact, cellular replacement of all alveolar tissue, as seen in the lower lobes, to small groupings of pleomorphic cells scattered between large patches of dilated, ruptured, and collapsed alveoli. The infiltrative process extended into the interstitial tissue adjoining the alveolar walls. The cysts were irregular in contour and seemed to be formed from a central breakdown of the denser cell masses. The cyst walls frequently were very wide, and were without a true epithelial lining. Most of the larger cysts were empty, and smooth-walled, as if air-containing. Others had roughened, even ruptured, walls and contained free, large mononuclear cells and neutrophils. Intact bronchial and vascular structures, enclosed by a thin collar of fibrous tissue, occupied some of the cyst cavities which sometimes were in direct continuity with alveolar spaces.

The cellular changes were pleomorphic, but the predominant cell was a large mononuclear histiocyte, measuring up to 30 μ in diameter. The nucleus was large, vesicular, well defined, and generally basophilic. As a rule the nuclei were round or ovoid, but some were notched, twisted, bent, or reniform. Nuclear chromatin sometimes was quite distinct, with a definite reticular pattern. In a few sites mitotic figures were detected. The cytoplasm was pale pink, occasionally finely granular, with irregular shape and indefinite margin, and usually with a smaller diameter than that of the nucleus. Frequently the cells were

tightly packed in massive sheet-like or nodular aggregations. Characteristic lipoid granules and doubly refractile bodies were not seen. Mingled with the histiocytic cells were varying numbers of neutrophils, eosinophils, lymphocytes, and a few giant cells. The last appeared to represent fusion of mononuclear cells, and they frequently contained phagocytized cell fragments. They varied in size from two to four times that of the average mononuclear cell. Fibroblasts were present in some areas. Although fibrous tissue was found in and around the more advanced lesions, it was seen also to accompany small groups of infiltrating cells.

Denuded mononuclear cells were present within a few alveoli, bronchioles, venules, and arterioles. Groups of these cells invaded and successfully penetrated bronchiolar, venous, and arteriolar walls. Generally, the lesions were extremely vascular. Occasional hemorrhages occurred into cyst cavities and walls, bronchioles, and alveoli. Numerous "heart lesion cells" were found in the alveoli and bronchioles.

Hilar Lymph Nodes. The germinal follicles of the hilar lymph nodes were reduced in size and number. They were compressed by marked proliferation of the cellular process within the sinusoids. Large mononuclear cells, numerous eosinophils, histiocytes, and giant cells were evident (Fig. 8). The capsules were invaded by the histiocytic cells, and were fused. Generalized swelling and neutrophilic infiltration occurred.

Skin. Re-examination of the original sections of skin taken for biopsy showed the typical mononuclear cells loosely scattered in the dermis. They were associated with a streaky increase in fibrous tissue, and often were in close proximity to small blood vessels.

Heart. Characteristic mononuclear cells were mingled with many polymorphonuclear cells in the heart and there was considerable avascular fibrous tissue in an irregular pattern throughout the subepicardial fat near the atrioventricular junction. The myocardial cells contained unipolar, golden-brown pigment granules, and those of the columnae carneae showed early hypertrophy.

Pituitary Gland. In the pituitary gland, the infundibulum and proximal portion of the pars nervosa and the capsule of the pars distalis were moderately infiltrated by many mononuclear cells, histiocytes, and lymphocytes. There was a great increase in the beta cells of the pars distalis.

Hypothalamus. Sections of the hypothalamus at anterior and posterior levels, including the mammillary bodies, third ventricle, and adjacent nuclei, showed localized collections of the large mononuclear

cells together with many lymphocytes and histiocytes, some eosinophils, and occasional giant and plasma cells. Considerable gliosis and vascularity were associated. The cells were gathered in and around the vessels, and at one point had eroded the wall of the third ventricle.

Spleen. Only a slight increase in capsular and trabecular fibers was seen in the spleen.

Kidney. Small foci of mononuclear and histiocytic cells were scattered in the pelvic mucosa of the kidney.

Other Viscera. The above organs and other viscera showed acute congestive changes. The liver, in addition, showed moderate atrophy of parenchymal cells and pigmentation in the central areas of the lobules.

Discussion

The case reported here was undiagnosed prior to the patient's death. Only after the histologic sections were reviewed did it become evident that the disease process was related to one of the three reticulo-endothelial disorders under discussion. In the past, the general rule has been to consider Letterer-Siwe's disease and eosinophilic granuloma of bone as conforming to a limited syndrome, while Christian-Schüller's disease has been recognized as having a variable clinical course and anatomical pattern. As a result, the atypical case, especially of Letterer-Siwe's disease, has sometimes been mistaken for Christian-Schüller's disease. Even though the atypical case may not fall clearly into any one of the three microscopic categories, it is the tendency at present to classify a new case on the basis of one of the three following general pictures.

Letterer-Siwe's disease follows an acute and malignant course and occurs predominantly in infants and children. Sex incidence is equal. The disease may exist for periods ranging from weeks to several years. There are no foam cells in the lesions save in occasional cases, and usually they occur in those of extended duration. There is a hemorrhagic tendency. The "Christian-Schüller triad" is rarely seen. Low-grade, continuous fever, moderate to severe secondary anemia, and a moderate leukocytosis or leukopenia appear. Lesions occur almost without fail in the spleen, liver, lymph nodes, skeleton, and skin, and occasionally may be found in the lungs, central nervous system, thyroid gland, gastro-intestinal tract[100] and other viscera, and in the thymus, an organ rarely involved by systemic disease. There is no response to treatment, and the disease invariably is fatal.

Eosinophilic granuloma of bone has a benign course. The general health remains good. The disease occurs predominantly in infants, children, and young adults under 20 years, although patients up to 58

years of age have been seen. The majority of cases are in males. The duration is brief, though sometimes the process will recur or become chronic. Only rarely are lipoid cells seen. Symptoms and signs are meager: some local tenderness, occasional weight loss, variable eosinophilia. Single or multiple bone lesions are present, and visceral extension is noted infrequently. Eosinophilic granulomatous lesions in the intestine have been reported,[101] but their relationship to eosinophilic granuloma of bone is doubtful. Lesions may develop and heal rapidly. The prognosis is good, as healing of bone lesions is spontaneous or follows surgical or roentgenologic treatment.

Christian-Schüller's disease is a chronic process, with insidious onset, and with extreme variation in course, localization, and clinical manifestations. The patient usually is an adult, although many instances in children are known. Either sex is involved. The disease lasts for many years. Lipoid cells are present at some time during the course of the disease. Clinical findings depend on the tissues involved, and are not specific, although exophthalmus and pituitary diabetes from bone lesions are frequent. Although the skeleton is the chief system attacked, almost any organ in the body can be damaged directly. The mortality is about 70 per cent. In the very young the disease may have a rapidly fatal course.

It is apparent that the case reported here does not resemble any of these descriptions. The disease, of insidious onset in a young male adult, pursued a chronic, progressively fatal course with severe symptoms terminally. The age, course, and lack of extension to organs such as the liver and spleen distinguished the process from Letterer-Siwe's disease. The absence of gross skeletal involvement and the presence of soft tissue lesions tended to rule out eosinophilic granuloma of bone. Renal, cardiac, cutaneous, and pituitary lesions pointed to Christian-Schüller's disease, despite the lack of skeletal changes. In course, symptomatology, age, and duration, the disorder was most like Christian-Schüller's disease, yet the lesions presented no detectable foam cells. Since the life history and the distribution of the lesions did not point to a definite diagnosis, it is necessary to seek the answer in histologic changes.

The three reticulo-endothelioses are histologically similar, with hyperplasia of macrophagic cells and no indication of anaplasia or suppuration. Common to all is the large (15 to 40 μ), pale, polygonal, sometimes round or elongated mononuclear cell, which is present in large numbers and is often phagocytic. The nucleus is large, single, vesicular, oval or indented, sharply outlined, and often eccentric. Mitotic figures and nucleoli are seen infrequently. The cytoplasm is

pale, dense, homogeneous, eosinophilic but occasionally basophilic or neutral, and frequently poorly outlined. Small multinucleated giant cells, with four to ten nuclei, sometimes phagocytic, occasionally are present. Reticulum fibers are increased. Small areas of necrosis and hemorrhage are seen, especially in the lymphoid tissues. These may be depleted, with resulting distortion of tissue architecture. Bone may be destroyed or occupied by cysts. Lymphocytes, plasma cells, and neutrophils may be seen.

Certain histologic features, when present, tend to distinguish the three syndromes. Eosinophilic granuloma of bone is found as a compact tumor enclosed by a fibrous and bony capsule, starting in the medulla and tending to expand and to erode. Letterer-Siwe's disease usually contains discretely arranged cells in nodular or diffuse formation. Christian-Schüller's disease is basically a nodular lesion, and foam cells are intermingled with mononuclear cells, or occur in compact masses. The nuclei may contain lipid droplets, or the cytoplasm, lipid crystals. If proliferative and granulomatous stages only are seen, as in an early lesion, foam cells may be absent and mononuclear cells and eosinophils predominate. Prominent sheet-like collections and masses of eosinophils are characteristic of eosinophilic granuloma of bone, although numerous eosinophils may occur also in the other two disorders. Eosinophils may disappear with increasing age of the eosinophilic granulomatous lesion, with conversion of the mononuclear cells to foam cells.

For the case reported here the histologic changes have been described. In summary, the predominant cell was a large mononuclear histiocyte with a vesicular basophilic nucleus. Small giant and other inflammatory cells were found. There were areas of necrosis and hemorrhage. The lesions varied from the young proliferative to the older fibrosing type. In places the reticular network was increased and altered. Doubly refractile bodies and foam cells were not found. Lymphoid tissue was reduced. Depending on the organ, the lesions had a nodular or diffuse character. In general, the microscopic findings resembled those noted in the reticulo-endothelial disorders under discussion, but they were most like those observed in Letterer-Siwe's disease. Yet, in the absence of lesions in the liver and spleen, they did not conform to the usual picture of that disease.

The organs involved were lung, pituitary body, hypothalamus, skin, heart, kidney, and lymph nodes. Pulmonary lesions have been described for all three syndromes, occurring as widespread, interstitial infiltrations. They tend to be fibrotic in Christian-Schüller's disease, and finely nodular in eosinophilic granuloma of bone. Nodulation is

common to all. Emphysema and polycystic pulmonary changes within nodular areas, and spontaneous pneumothorax, are recognized.[45,60,72,102]

Direct invasion of the pituitary body by the disease process, rather than pituitary compression from surrounding bone lesions, produced diabetes in our patient. The invasion seems to have extended from the older hypothalamic sites along the infundibular stalk into the pars nervosa. True involvement of these structures has been described frequently in Christian-Schüller's disease, occasionally in Letterer-Siwe's disease,[61,75] and rarely in eosinophilic granuloma of bone.[30,103,104] The combination of pituitary and pulmonary lesions is well known in Christian-Schüller's disease,[12,23,98] but it has been described also in eosinophilic granuloma of bone [68] and in Letterer-Siwe's disease.[105]

As noted previously, skin, lymph nodes, epicardial fat, and renal pelvis were additional sites of lesions in our case. Cutaneous lesions are the rule in Letterer-Siwe's disease, and occur frequently in Christian-Schüller's disease. In acute cases of either syndrome, the lesion usually is petechial in appearance, while in chronic cases it is often seborrheic, papular, scaly, crusted, isolated, or diffusely distributed in small groups. The histiocytic cells accumulate in the corium, often perivascularly. Lesions of lymph nodes have occurred regularly in Letterer-Siwe's and Christian-Schüller's diseases, and have been reported in eosinophilic granuloma of bone.[95] Lesions in epicardial fat have been noted in Christian-Schüller's disease,[21] and in Letterer-Siwe's disease.[61,76] Changes have been observed also in the renal pelvis in Letterer-Siwe's disease.[61,75,76,102]

The differential diagnosis of the diseases under discussion should include a consideration of several other disorders. The lesions of Hodgkin's disease,[25,106,107] mycosis fungoides,[108,109] monocytic leukemia,[25,110] tuberous sclerosis,[105] Boeck's sarcoid,[111] multiple myeloma,[112,113] xanthoma tuberosum,[114] and other diseases,[115-120] sometimes may resemble the changes noted in our case.

The pathogenesis of the pulmonary cysts is of interest. Oswald and Parkinson[105] have explained these lesions on the basis of impeded expiration due to bronchial obstruction, and stress in the more normal parts of the lungs after widespread alveolar destruction elsewhere. Pulmonary cysts may occur in many disorders with underlying pulmonary damage. Such patients experience progressive dyspnea and spontaneous pneumothorax. On roentgenologic examination, a fine or coarse reticular pattern is seen throughout the lung. The media for bronchography fail to enter the cysts, even after deep coughing. As a rule, younger patients will die of spontaneous pneumothorax and older ones of right heart failure without spontaneous pneumothorax. A number

of patients with reticulo-endotheliosis [5,17,21,27,105,121] have died of heart failure secondary to pulmonary fibrosis.

Summary and Conclusions

A case of a granulomatous disease involving the reticulo-endothelial system forms the basis of this report. After reviewing the pertinent literature on three major reticulo-endothelial granulomatous diseases, namely, Christian-Schüller's disease, Letterer-Siwe's disease, and eosinophilic granuloma of bone, and comparing the clinical and pathologic aspects of our case with those of each of these three syndromes, it was found to resemble Letterer-Siwe's disease more closely than it did either of the other two.

We concur with the view that the three syndromes represent the more frequently encountered clinical variants resulting from a single fundamental disorder of the reticulo-endothelial system. This view is adopted chiefly because of the histologic similarities among the three diseases, with full recognition of their varying clinical patterns and prognoses.

Many investigators who accept the doctrine of three distinctly different entities, base their position mainly on this variation in clinical course and prognosis. However, it is our impression that differences in the distribution and location of lesions, rather than differences in the fundamental disease process, are the chief determinants of clinical course and prognosis. Also of importance in influencing clinical course are differences in host susceptibility related to age and to other unknown factors.

With the assumption that the three usually accepted disease pictures represent a single, fundamental pathologic change in the reticulo-endothelial system, it becomes possible to explain the great number of atypical cases which do not fall clearly into any one of the three patterns, and which therefore have been the subject of controversy. Greater awareness of the atypical case will no doubt result in a more comprehensive understanding of the basic disease process.

This hypothesis suggests that extreme variations in clinical course are due in part to alterations in host or organ resistance paralleling the ageing process. Perhaps the fulminating Letterer-Siwe's disease in a child and the chronic progressive manifestations of the Christian-Schüller's variant in an adult can both be explained partially on this basis.

The thesis of a fundamental pathologic disorder which is responsible for the three accepted clinical variants and also for the numerous atypical cases which do not follow the prescribed pattern of these

variants suggests the advisibility of abandoning the present eponymic designations. Instead it is proposed that there be adopted a single, all-inclusive term which gives cognizance to the currently unidentified etiologic factor or factors. Such a designation is primary reticulo-endothelial granuloma.

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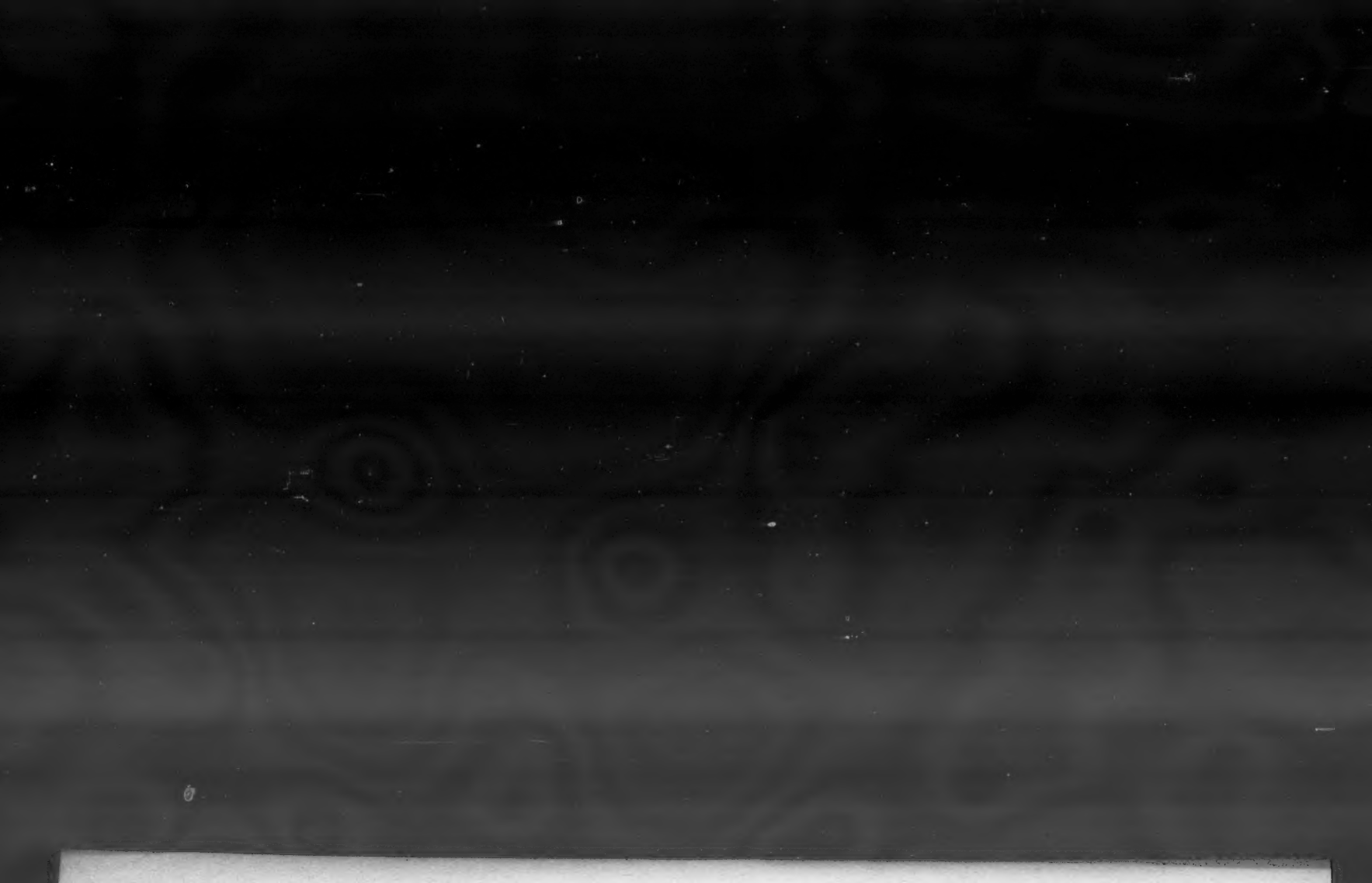
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 113

FIG. 1. Chest plate taken on June 8, 1946, showing no essential changes in lung fields.

FIG. 2. Bronchogram of right lung made on February 9, 1948, interpreted as showing good filling and some displacement of the middle and lower lobe bronchi, and no bronchiectasis.



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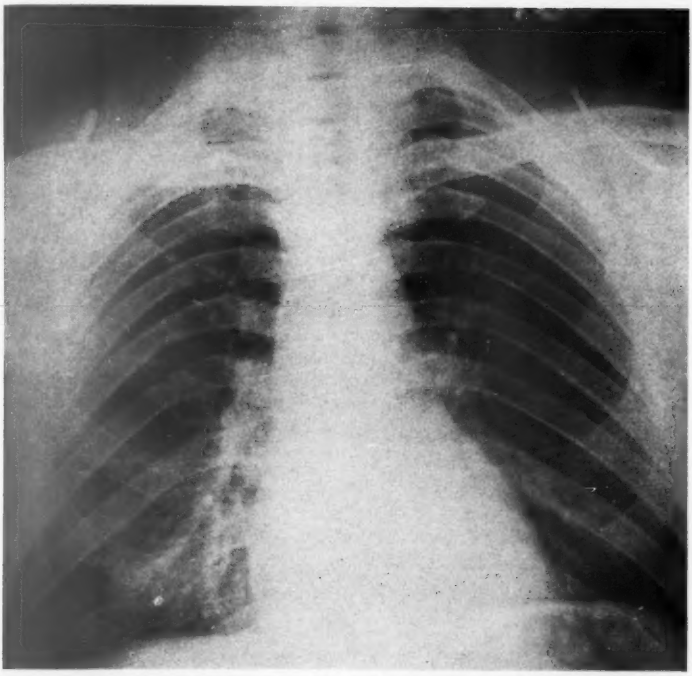

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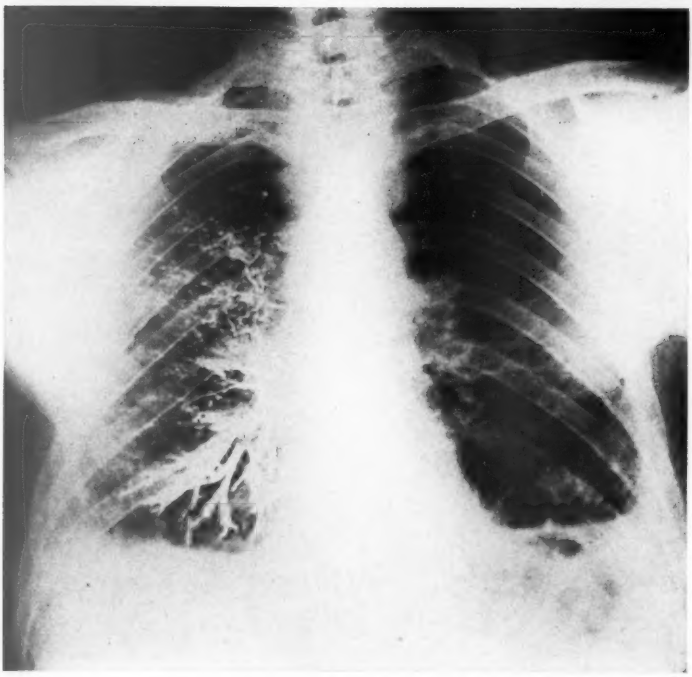
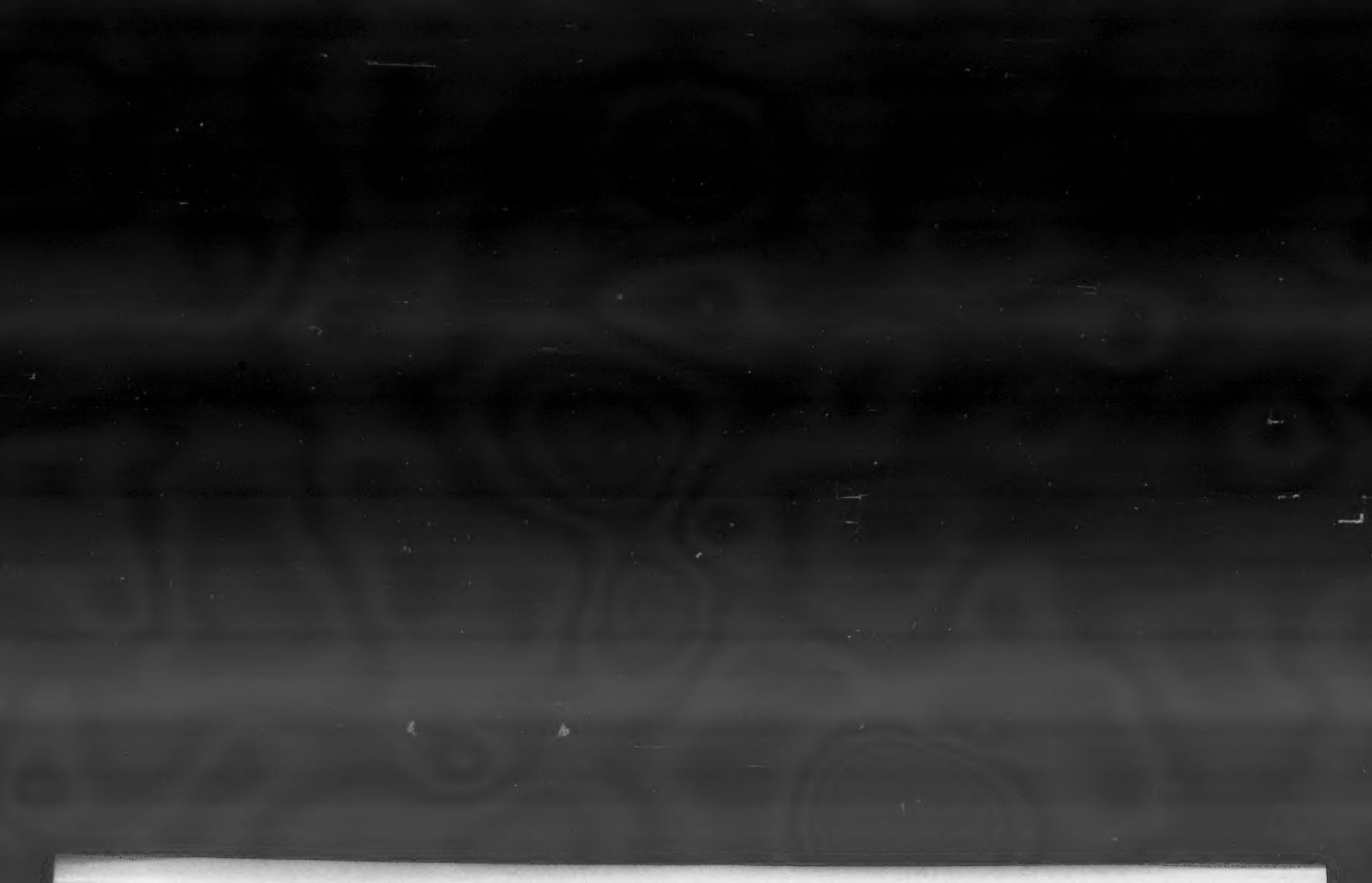


Dennis and Rosahn Primary Reticulo-endothelial Granulomas

PLATE 114

FIG. 3. Cut surface of lung showing characteristic pulmonary changes. This and the following photograph were taken after fixation in formalin preservative.

FIG. 4. Higher magnification of portion of Figure 3 showing detail of cystic and compact areas.



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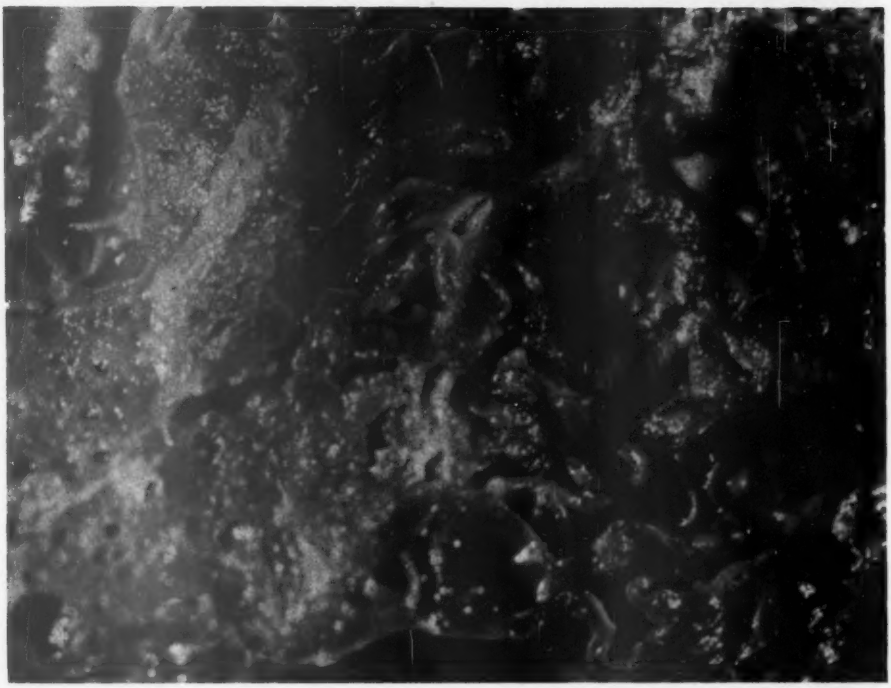

Dennis and Rosahn Primary Reticulo-endothelial Granulomas

PLATE 115

FIG. 5. Bronchiole with desquamated cells in lumen and cellular infiltrate in surrounding tissue. Hematoxylin and eosin stain. × 250.

FIG. 6. Wall of pulmonary cyst, showing absence of cell lining, and pleomorphic cellular infiltrate. Hematoxylin and eosin stain. × 250.

FIG. 7. Detail of characteristic cells in pulmonary lesion. Hematoxylin and eosin stain. × 1200.

FIG. 8. Cellular changes in lymph nodes. Hematoxylin and eosin stain. × 462.

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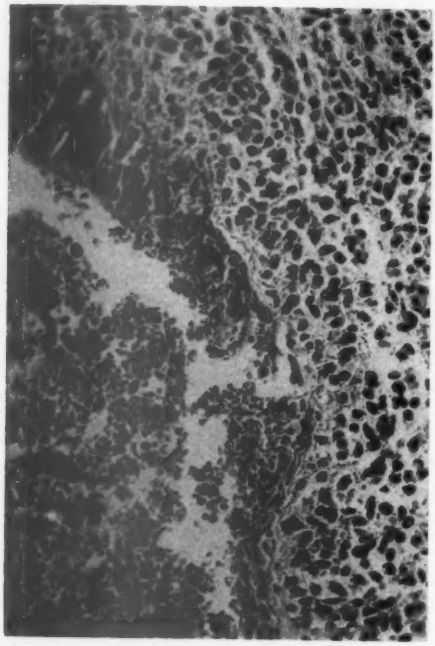

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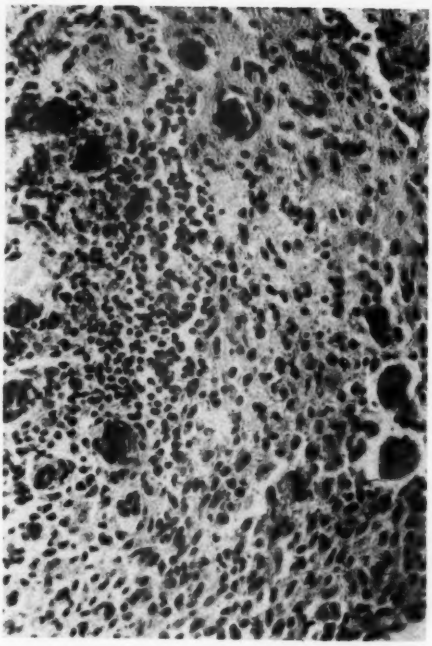

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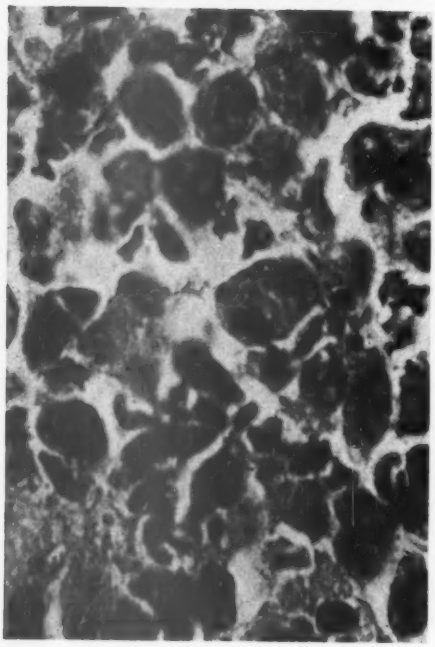

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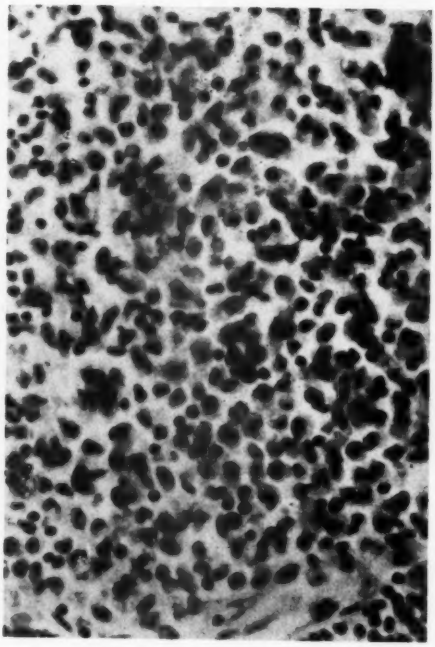

Dennis and Rosahn

Primary Reticulo-endothelial Granulomas

TOXOPLASMOSIS OF CAPTIVE WILD BIRDS AND MAMMALS *

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Toxoplasmosis is best known as a human disease affecting infants and children, and occurring much less frequently in adults. In the young it nearly always manifests itself as a meningo-encephalitis which usually ends fatally within a few weeks.[1-5] In adults, the symptoms of the few recorded cases have suggested Rocky Mountain tick fever, and evidence of generalized infection was found by post-mortem examination.[6,7] A number of species of animals are known to be susceptible to the naturally occurring form of this infection. In them the central nervous system of both young and adults is predominantly involved [8-13]; less frequently, lesions have been found also in lymph nodes, liver, and lungs.[14-17] When the disease is induced experimentally, either in mammals or birds, the central nervous system likewise is the site most commonly attacked, even though the organisms are introduced into other regions.

In contrast to the predilection for the nervous system in most hitherto reported cases of toxoplasmosis of either man or lower animals, we have found involvement of various other organs to be the dominant feature in 27 consecutive fatal examples of toxoplasmosis encountered during the past 10 years at the Philadelphia Zoological Garden. This paper deals with the epidemiology, the distribution and appearance of the disease processes, and the morphologic character of the parasites in these animals.

Material and Methods

The present series comprises 13 mammals and 14 birds which died during the period 1940 to 1950. All of the birds and 2 of the mammals were newly imported adults; none of them lived more than 4 months after arrival. Nine other mammals were adults, and had been exhibited for periods ranging from 10 months to 5 years. Two mammals, which were immature, were born in the Garden. In the majority of these animals the disease progressed to fatal termination without clinical signs of illness having become obvious. In fact, the nature of the disease usually was not recognized until tissues were examined microscopically. Since sections, as a rule, were made only from organs which were macroscopically abnormal, the distribution of the lesions,

* Received for publication, August 4, 1950.

listed for this series in Table I, must be considered somewhat incomplete.

All tissues were fixed in either formaldehyde or Bouin's solution, and embedded in paraffin. Sections were cut at 3 to 4 μ and stained with hematoxylin and eosin, and by methods suggested by Perrin [18] for differentiating Toxoplasma from Encephalitozoon in tissues. Smears of tissues were stained by Giemsa's method.

Epidemiologic Survey

This series of animals has been arranged, in Table I, according to the chronologic sequence of their deaths. The first column gives the genus and species, and its common name. Exhibition periods, stated in the third column, are the intervals between arrival or birth, and death. Duration of illness, in the fourth column, is the interval between the recognition of illness and death. Organs listed as containing lesions are those in which definite necrosis and inflammation were associated with Toxoplasma infestation. Organs containing the parasites in cytoplasmic cysts within parenchymal cells, but exhibiting no other signs of tissue damage, are not included in this table.

The first animal of this series, a snow leopard, which died 2 years before the second animal, represents an isolated example of the disease both in its exhibition area and taxonomic group. In it, also, the disease apparently had progressed less rapidly than in any of the others.

Animals 2, 3, and 4 had all lived in one building, the Small Mammal House; all died of toxoplasmosis within a period of 4 months. Two, both squirrel monkeys (cases 2 and 3 in Table I), had been cage mates.

Four years later, in 1946, within a period of about 3 months, 5 additional deaths from toxoplasmosis occurred. One of these (case 6) also came from the Small Mammal House. The 4 penguins, *Spheniscus humboldti* (cases 5, 7, 8, and 9), had never been in contact with any of the other animals in the series. They were members of a group of 9 penguins, all of which had been received from a dealer at the same time, and which had been exhibited together in a shaded outdoor enclosure containing a large pool. The remaining penguins of the group died within 1 year after arrival, but no evidence of toxoplasmosis was found in any of them.

During the following year, 1947, 8 animals died of toxoplasmosis. The first, in chronologic order, was an infant sea lion (case 10), 10 days of age. Its mother was one of a group kept in a large outdoor pool; the number of its inhabitants has varied over the past 10 years with new arrivals, births, and deaths. Thus far, 43 sea lions have died

and have been examined post mortem, but no other examples of toxoplasmosis have been encountered. The spider monkey (case 11) was one of a group of 6, all of which were purchased at the same time and exhibited in one cage. Its fellows have remained healthy. The next animal (case 12) was one of a group of 14 penguins of various species, all received in May, 1947. The 13 remaining members of this group have since died and have been examined post mortem, but none was found to have been infected by Toxoplasma. The crowned pigeon (case 13) was one of a pair which were received and exhibited together. Its cage mate is living and apparently healthy at the present time.

In the above 4 fatal cases of toxoplasmosis no common source of infection was traceable, but in the following 4 cases it is highly probable that the disease was directly or indirectly transmitted. Three animals, two tree porcupines and a hyrax (cases 14, 16, and 17), were exhibited in the Small Mammal House where 4 of the earlier infections had developed. The wallaby (case 15), while not maintained in this building, was fed several times each week by an attendant who also worked in the Small Mammal House.

During 1948, the 2 fatal infections also probably were related; a second wallaby (case 18) was fed by a keeper also on duty in the Small Mammal House in which the woodchuck (case 19) was exhibited.

A period of almost 11 months then elapsed before the next examples of the disease were encountered. Then, within a few days, 8 penguins died of toxoplasmosis. These are listed as cases 20 to 27 in Table I. Cases 20 to 26, inclusive, were black-footed penguins, all of which appeared to be healthy upon arrival in the Zoological Garden on July 5, 1949. They continued to be well for only 1 week; then one of the birds refused food and became inactive. It was isolated and hand fed, but died 3 days later. In rapid succession others of this group also failed to eat, became inactive, and died 2 to 4 days thereafter, the last, 21 days after arrival. These birds were kept on a small pond where the other penguins listed in Table I had been maintained. At the time of their arrival in July, 2 penguins of other species, *S. magellanicus* and *S. humboldti,* were present in this enclosure. The first of these had been on exhibition since April 19, 1949, during which time it had behaved normally and fed regularly. It became ill on August 23, 1949, and died 3 days later, that is, the same day on which the last of the black-footed penguins succumbed. The other penguin, *S. humboldti,* a resident of this pond for about 2 years, has continued in good condition.

TABLE I

Mammals and Birds in Which Toxoplasmosis Has Been Recognized at the Philadelphia Zoological Garden, Arranged in the Sequence of Their Deaths, with Exhibition Periods, Duration of Recognizable Illness, and Distribution of the Lesions

Animals	Date of death	Exhibition period	Duration of illness	Distribution of lesions					
				Heart	Lungs	Liver	Spleen	Abdominal lymph nodes	Other organs
		months							
1. Snow leopard, *Felis uncia*	2-26-40	21	2 weeks		+	+		+	Adrenals, serous surfaces
2. Squirrel monkey, *Saimiri sciurea*	9-29-42	47	None	+	+	+	+	+	Hemorrhagic necrosis of adrenal cortex
3. Squirrel monkey	11-3-42	58	None		+	+			
4. Viscacha, *Viscacha viscacha*	2-2-43	17	None	+	+	+			
5. Humboldt's penguin, *Spheniscus humboldti*	8-9-46	0.3	None			+	+		
6. Mountain chinchilla, *Lagidium peruanum*	9-10-46	0.2	None	+	+	+	+		
7. Humboldt's penguin	9-11-46	1	2 days		+	+	+		
8. Humboldt's penguin	11-19-46	3	None	+	+	+	+		
9. Humboldt's penguin	11-29-46	3	6 days		+	+	+		
10. Sea lion *Zalophus californianus*	6-9-47 (newborn)	0.3	None	+	+	+		+	
11. Spider monkey, *Ateles geoffroyi*	8-2-47	10	3 days			+		+	Granuloma of the pancreas

12. Penguin, *Spheniscus magellanicus*	8-18-47	3	None		+	+			
13. Crowned pigeon, *Gura victoria*	10-11-47	4	None		+	+	+		
14. Tree porcupine, *Coendou prehensilis*	10-13-47	61	2 weeks		+	+	+		
15. Wallaby, *Macropus bennetti*	10-14-47	14	None	+	+		+	+	
16. Cape hyrax, *Procavia capensis*	10-18-47	2	None		+	+	+		Mononuclear cells increased in meninges
17. Tree porcupine	10-24-47	61	2 weeks		+	+	+		
18. Wallaby	8-19-48	6	None	+	+				
19. Woodchuck, *Marmota monax*	8-24-48	24	None	+	+				
20-26. 7 Black-footed penguins, *Sphenicus demersus*	7-15-49 to 7-26-49	0.2 to 0.5	2-4 days		+	+	+		Combined with lesions of acute hepatitis
27. Penguin, *Sphenicus magellanicus*	7-26-49	4	4 days		+	+	+		Combined with lesions of acute hepatitis

In brief, 22 of the 27 deaths in this series could be accounted for by five separate outbreaks of the disease. In each of these outbreaks there was a high degree of probability that the infection had spread from a common source. However, this cannot be established definitely. Among these probable examples of group infection the susceptibility of penguins has been outstanding, one group of 7 having been exterminated within 3 weeks and, in another, 4 of 9 dying during a period of 3 months. Among the mammals, 2 squirrel monkeys and 2 tree porcupines were cage mates, and 5 others were closely associated or were fed by the same attendants. By contrast, evidence of a common source of infection was lacking for 5 animals; moreover their cage mates and close associates remained free of demonstrable infection.

Description of the Disease Processes

At necropsy the macroscopic appearance of the viscera was not distinctive in any of the animals. The changes most commonly encountered were dilatation of the heart, often associated with focal necrosis of the myocardium; pronounced edema of the lungs; intense congestion of the liver, sometimes with widely distributed necrotic foci; and hyperplasia of the spleen. Occasionally the mesenteric lymph nodes were enlarged, but otherwise unchanged. The brains of 8 mammals and 3 birds were examined carefully without finding evidence of infection; in the remaining 16 animals of the series no examination of the brain was made.

The one exception to this disease pattern was encountered in the snow leopard (case 1). In this animal the infection was associated with acute pleuritis and peritonitis, by enormous enlargement and caseation of mesenteric and retroperitoneal lymph nodes, and by fibrosis and caseation of the adrenals. The lungs were subcrepitant and contained poorly defined, soft nodules. The spleen was large and firm, the liver was congested, and the heart was dilated. The appearance of the organs suggested tuberculosis, but bacilli could not be found.

Microscopically, the changes in the tissues lacked variety. This was especially true of the lesions in the lungs. These were present to a greater or less degree in all animals, and consisted of patchy, or widely spread thickening of the alveolar walls and interlobular septa due to edema and infiltration of mononuclear leukocytes. The vessels usually were intensely injected (Figs. 1, 2, and 3). Interstitial inflammation in the lungs was not proportional to the number of Toxoplasma found in the sections. The parasites were never as abundant as in other organs, and in some animals they could not be demonstrated readily in sec-

tions. Fortunately, in 2 such cases contact smears of the cut surfaces were available for comparison; in them the parasites were seen readily, usually within cells.

In the heart, liver, spleen, and lymph nodes of the animals listed as cases 1 through 19, the lesions were closely similar. In the more acute stages they consisted mainly of foci of necrosis, varying in diameter from about 200 μ to 2 to 3 cm.; they were distributed at random throughout the organs. The smaller lesions were composed of compact, eosin-staining tissue remnants and nuclear fragments, about which had accumulated sparse mononuclear and polymorphonuclear leukocytes. Larger foci were expansions of smaller ones, with the central necrotic mass proportionately larger, and surrounded by a loosely arranged zone of leukocytes and macrophages, often imperfectly preserved and mixed with degenerated tissue (Fig. 4). In the more chronic lesions, the necrotic foci had been replaced by fibroblasts and macrophages.

The number of Toxoplasma found in the necrotic lesions in the heart, liver, spleen, and lymph nodes was roughly proportional to the size of the necrotic foci. The smaller foci usually contained relatively few organisms, and these lay within the degenerated tissue elements, singly or in small groups. In the larger foci, the organisms usually were abundant, and also often numerous in parenchymal cells and macrophages adjacent to the necrotic foci (Fig. 4). In tissue invasion of this type, the organism did not form cytoplasmic cysts. In the foci where macrophages and fibroblasts had replaced the necrotic tissue more or less completely, the parasites were present only in small numbers (Fig. 5).

The lesions in the 8 penguins listed as cases 20 through 27 were somewhat different from those of other members of the series. Obvious necrosis of the liver, spleen, or myocardium was not present. Instead, the vascular bed of the liver and spleen was packed with phagocytic cells, many of which contained incompletely or completely segmented masses of organisms which corresponded, in their staining reactions, to Toxoplasma. The hepatic and myocardial cells were not invaded by the parasite (Figs. 6 and 7). The reactions in the lungs, however, closely resembled those described above. It seems likely that in this group of birds the infection was more fulminant than in the other members of the series.

The Parasite

The organisms identified as Toxoplasma have been studied in sections of tissues from all of the 27 animals, and in contact smears from

one or more organs of 2 mammals and 8 birds. Except for the greater ease with which organisms were found in the smears, there was no essential difference between their appearance in smears and in sections from the corresponding organs. There were, however, considerable differences in the shape and size of the organisms from animal to animal. On the basis of morphology there appeared to be three distinct types or stages of Toxoplasma. The most common form was of relatively large size, 5 to 8 μ in length and about 3 μ in width. The second form was similar in shape, but distinctly smaller, 4 to 5 μ in length and about 1 μ in width. The third appeared as masses of incompletely segmented cytoplasm in which imperfectly formed, small crescents could be seen, or as masses of unsegmented cytoplasm containing large numbers of tiny nuclei.

The smaller crescents were especially numerous in smears and sections of tissue from the crowned pigeon (case 13), a wallaby (case 15), and a tree porcupine (case 17). However, in these animals, as well as in occasional others of the series, the small crescents occurred together with the larger ones, but intermediate stages were never encountered, nor did the two forms ever exist within the same parasitized cells.

The plasmodial type varied in size from 10 to 100 μ in diameter. The cytoplasm stained well with basic dyes; the nuclei appeared as solidly stained, darker masses about 1 μ in diameter. In one animal, the tree porcupine (case 17), plasmodia were associated with small crescentic parasites. In the penguins (cases 20 to 27) they were greatly predominant, although both large and small crescents also were present.

It is not yet possible to express an opinion concerning the relationship of these three forms; they may be unrelated species, or they may be different stages of the same parasite.

Discussion

Organisms of the genus Toxoplasma are known to be widely distributed and relatively common parasites of many species of mammals and birds.[5] Active toxoplasmosis, however, appears to be an uncommon disease, and usually has developed as a subacute or chronic process. With one exception the disease in this series of animals was acute, and the lesions corresponded closely to toxoplasmosis of adult human beings.

The conditions under which Toxoplasma become virulent parasites are not known. One may postulate that the organisms initiate disease only when their hosts are weakened by malnutrition or concurrent infection and consequent interference with the immune mechanism.

This supposition is supported by some reports in the literature,[6,9,10] but, more often, factors that might have reduced the health of the host have not been obvious.[5] In the present series, there were some animals in which toxoplasmosis was clearly associated with another disease. The crowned pigeon (case 13) and the penguins (cases 20 through 27) apparently had acquired infection by the virus of avian hepatitis. But in all other animals careful consideration leaves us unable to suggest factors which could have contributed to susceptibility toward the organism. It is possible that more than a single type of organism was encountered, although this is still conjectural. A better understanding of toxoplasmosis must await more complete knowledge of the life history of the parasite, and of the conditions under which it becomes virulent.

Summary

During a period of the past 10 years, 13 mammals and 14 birds developed fatal toxoplasmosis at the Philadelphia Zoological Garden. The disease is relatively rare, constituting less than 1 per cent of all deaths.

With one exception, the form of toxoplasmosis encountered was acute, and in all instances involved organs of both the thoracic and abdominal cavities. Clinical signs of localization of the organisms in the central nervous system were lacking. The brains of 8 mammals and 3 birds of the series were closely examined at necropsy, and no lesions were found.

The predominant tissue changes were foci of necrosis in liver, spleen, heart, lymph nodes, and lungs. The more acute lesions were accompanied by infiltration of monocytes. In less acute lesions, monocytes and fibroblasts largely replaced the necrotic tissue. In the lungs, diffuse interstitial infiltration of monocytes and edema usually characterized the disease, whether or not focal necrosis of the tissue had developed.

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DESCRIPTION OF PLATES

PLATE 116

FIG. 1. Lung, tree porcupine (*Coendou prehensilis*). This field illustrates the thickened alveolar walls and the increased numbers of alveolar macrophages which seemed to be part of the reaction of many animals of this series to infection by Toxoplasma. × 200.

FIG. 2. Lung, tree porcupine (*Coendou prehensilis*). This field includes a part of an alveolar wall, the surface of which is covered by macrophages, some of which contain carbon particles. Toxoplasma are scattered through these cells, and in the upper part of the field a large mass of these organisms lies within the outlines of a distended cell. × 800.

FIG. 3. Lung, penguin (*Spheniscus demersus*). This field includes the wall of a secondary bronchus, recognizable here by three bits of smooth muscle at the top of the field. These bands of smooth muscle surround passages through which air moves into alveolar spaces in the bird. These passages and the alveolar spaces are filled by mononuclear leukocytes. These cells also are abundant in the capillaries and larger blood vessels in this field. This view illustrates the most severe form of pneumonitis associated with toxoplasmosis of birds of this series. × 200.

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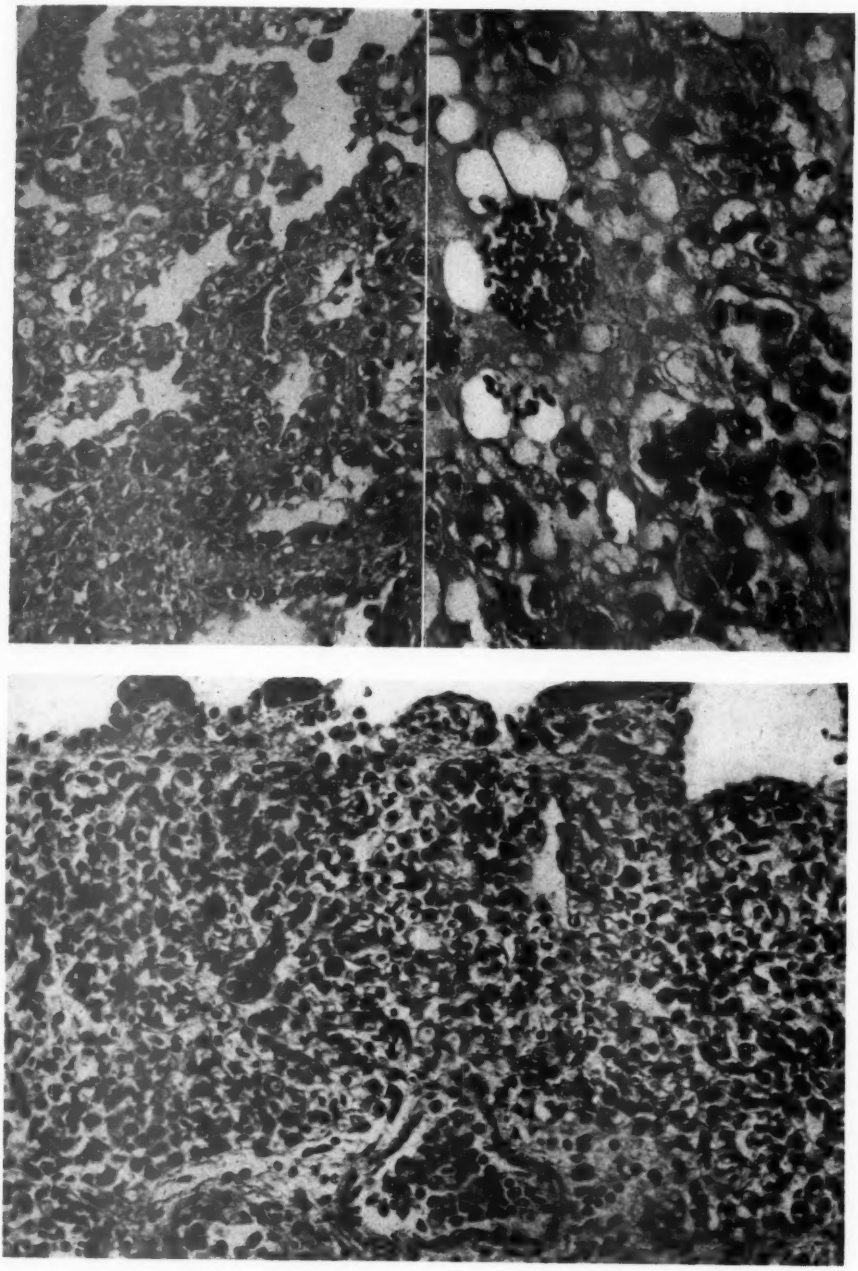


Ratcliffe and Worth Toxoplasmosis of Captive Animals

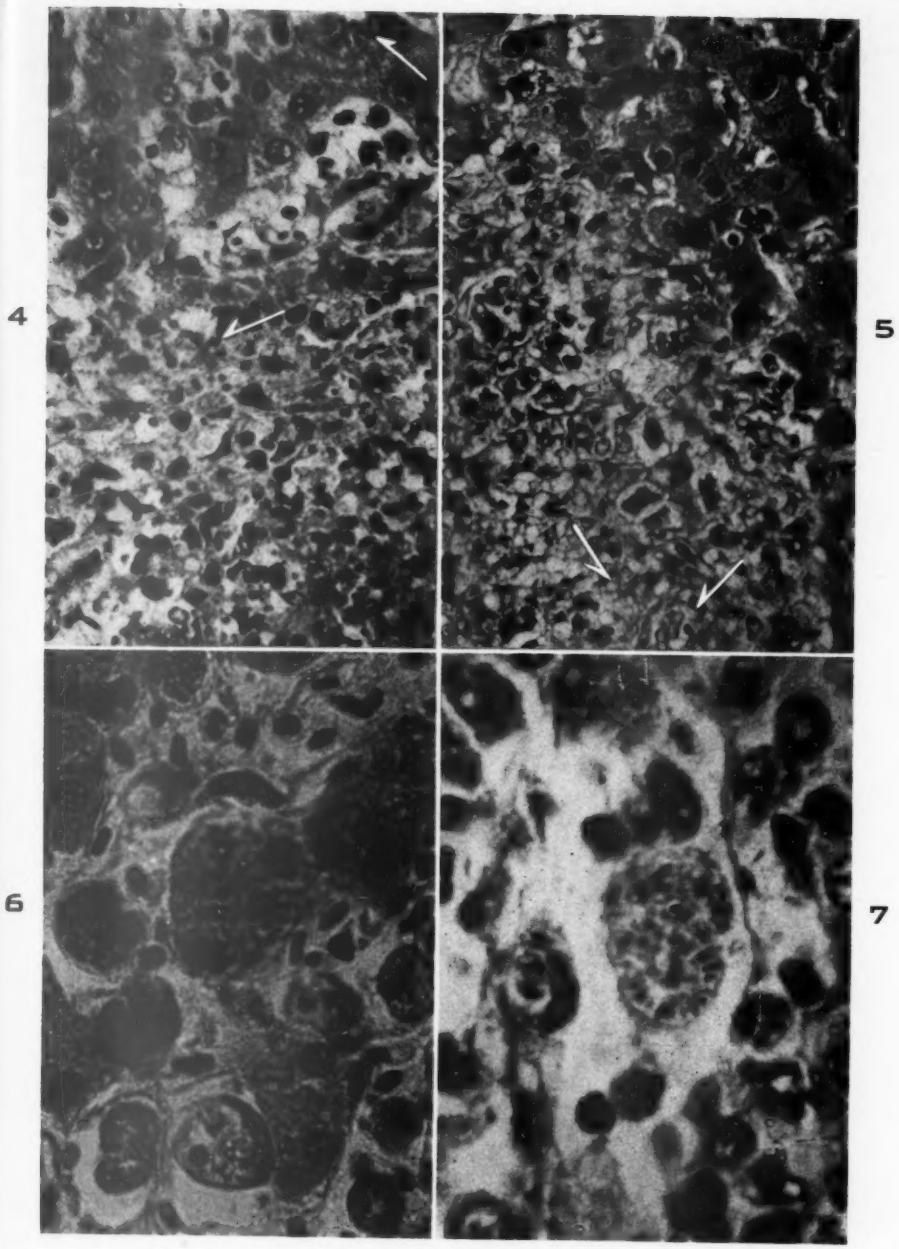
PLATE 117

FIG. 4. Liver, penguin (*Spheniscus humboldti*). This field includes part of a necrotic focus and its border of liver cells. Arrows point to Toxoplasma, which are numerous among the tissue fragments of the lesions, and to one small group of organisms which may be seen in the cytoplasm of a cell near the upper right border of the field. × 600.

FIG. 5. Liver, wallaby (*Macropus bennetti*). This field includes part of a lesion in which macrophages and fibroblasts replaced the parenchyma. Arrows point to small numbers of Toxoplasma in clear spaces within these cells. × 800.

FIG. 6. Liver, penguin (*Spheniscus demersus*). This photomicrograph illustrates the hyperplasia of Kupffer cells in the sinusoids of the livers of penguins no. 20 to 26 of Table I. Six of the Kupffer cells in this field contain various forms of the organism. × 1000.

FIG. 7. Liver, penguin (*Spheniscus demersus*). A mass of the small, crescent-shaped forms of Toxoplasma, such as is shown in the lower left field of Figure 6, is photographed here from a section cut at 2 μ and stained by iron hematoxylin. In this cell the small crescents were arranged radially about a jumbled mass of material. × 1700.



Ratcliffe and Worth Toxoplasmosis of Captive Animals

FORTY-EIGHTH ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

CLEVELAND

APRIL 26TH, 27TH, AND 28TH, 1951

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Eighth Annual Meeting,
Academy of Medicine,
Cleveland, Ohio
April 26th, 27th, and 28th, 1951

PRESIDENT MALLORY IN THE CHAIR

BUSINESS MEETING

April 26th, 1951

Upon nomination of the Council, the Association elected the following officers:

President	ROBERT A. MOORE
Vice-President	WILLIAM H. FELDMAN
Secretary	ALAN R. MORITZ
Treasurer	SIDNEY FARBER
Incoming Member of Council	EDWIN W. SCHULTZ

The President announced that the following officers had been elected by the Council:

Assistant Secretary	HERBERT Z. LUND
Assistant Treasurer	WILLIAM A. MEISSNER

For the Council, the President announced the following actions:

Election of New Members

Howard J. Barrie, Toronto
Chauncey G. Bly, Rochester, N.Y.
Earl W. Cauldwell, Chicago
George W. Changus, Memphis
John M. Craig, Boston
John Denst, Denver
John T. Ellis, New York City
Joseph E. Flynn, New York City
Alvan G. Foraker, Atlanta
Dorin L. Hinerman, Ann Arbor
Helen Ingleby, Philadelphia
Sidney D. Kobernick, Montreal
Joseph J. Lalich, Madison
Crichton McNeil, Salt Lake City
Herbert Mescon, Philadelphia
F. K. Mostofi, Washington
Robert W. Mowry, Bethesda
Ward M. O'Donnell, Lancaster, Pa.
J. Lowell Orbison, Cleveland
Torrence P. B. Payne, Newburgh, N.Y.
John T. Prior, Syracuse
James W. Reagan, South Euclid, Ohio
John W. Rebuck, Detroit
Theodore Robertson, Port Washington, N.Y.
Kurt Stern, Chicago
Helene W. Toolan, New York City
Raymond Yesner, Newington, Conn.

Acceptance, with regret, of the resignations of Drs. Carl D. Camp, William Dock, Isaac H. Erb, Thomas Francis, Jr., Raymond H. Goodale, William C. MacCarty, Sr., Frank R. Menne, Sidney J. S. Peirce, and Ralph P. Smith.

With deep regret, the recording of the deaths of Drs. William D. Collier, Leon S. Lippincott, Lawrence R. Morrison, George M. Smith, George A. Walker, and Francis C. Wood.

The re-election of Dr. Malcolm H. Soule as Assistant Editor of *The American Journal of Pathology* for the ensuing year, and the election of Dr. Webb E. Haymaker to the Editorial Board for a period of six years.

It was moved and seconded that, in consideration of the increasing costs of operating the Secretary's and Treasurer's offices, of preparing for and holding the annual scientific sessions, and of the publication of the Journal, the Council consider the feasibility of increasing the dues paid by members to the Association and deal realistically with the financial situation. This motion was passed by unanimous vote.

The President announced that the next meeting of the Association will be held in New York City on April 10, 11, and 12, 1952. The topic for the symposium is "Pathologic Aspects of Reactions to Ionizing Radiation." Dr. Shields Warren has been invited to act as referee.

REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a certification from Frank D. Flynn, Auditor, Melrose, Massachusetts. In condensed form, the Treasurer's report follows:

General Checking Account

Receipts

Balance on hand, January 1, 1950		$ 2,908.36
Membership dues	$ 7,827.20	
Interest on bonds, from investment account	500.00	
		8,327.20
		$11,235.56

Disbursements

American Journal of Pathology ($8.00 per member)	$ 7,512.00	
C. E. Lennon (Secretary to Dr. Moritz)	150.00	
H. McGachie (Secretary to Dr. Farber)	150.00	
Reporting 1950 meeting	338.87	
Officers' expenses at meetings (including printing, badges, travel)	465.60	
Auditing services	35.00	
General office expense, secretary	245.05	
General office expense, treasurer	130.48	
		$ 9,027.00
Balance on hand, December 31, 1950		$ 2,208.56

Investment Account

Balance, January 1, 1950	$34,688.78
Interest on bonds, 1950	500.00
Interest from savings banks	328.06
	$35,516.84
Transfer to checking account (bond interest)	500.00
Balance, December 31, 1950	$35,016.84

Inventory

U.S. bonds, series G.	$20,000.00
The Provident Institution for Savings	4,186.60
Franklin Savings Bank	4,183.21
Cambridge Savings Bank	4,486.25
National Shawmut Bank	2,160.78
Total, December 31, 1950	$35,016.84

EXPERIMENTAL TRANSFUSION OF BONE MARROW EMULSION INTO RABBITS AFTER TOTAL BODY IRRADIATION. Martin F. Hilfinger, Jr. (by invitation) and J. Howard Ferguson, Syracuse, N.Y.

Abstract. Studies were carried out on rabbits receiving intravenous bone marrow emulsion obtained from the femurs of other normal rabbits. The recipient animals received single total body irradiation from a 200 kv. machine at levels of 1000, 1200, and 1400 r. Three days after exposure the animals were transfused intravenously with bone marrow emulsified in the donor rabbit's own serum or plasma. The marrow emulsion ranged in cell count from 290 million to 1,590 million per cmm. As previously reported by others, the injected cells rapidly disappeared from the circulating blood. A control group receiving the same amount of radiation was run concurrently, the animals receiving no marrow emulsion. Results to date indicate that there is a considerably more rapid return of the white blood cells, both neutrophils (amphophils) and lymphocytes, toward pre-irradiation levels in those rabbits receiving the marrow emulsion. Studies are in progress to determine what effect this experimental procedure may have on the expected mortality.

Discussion

(Dr. Jacob Furth, Oak Ridge, Tenn.) Was there any protection in your series as concerns lethal effect (LD_{50})?

(Dr. Kornel Terplan, Buffalo, N.Y.) Were any studies made as to the rate of recovery of the intestinal epithelium in the control and injected animals?

(Dr. Cornelia Hoch-Ligeti, Charlottesville, Va.) Has any material other than bone marrow been used?

(Dr. Hilfinger) Was there any protection evident as far as mortality goes? Of the control animals, 2 died, these dying at about 2 weeks. Of the 5 injected animals, all of them now past 6 weeks, none has died as yet; they are all maintaining their weight; their counts are rising to rather high levels; most of them after 6 weeks will go from 20,000 to 30,000, the predominant cell being the lymphocyte.

In response to the second question, we have not studied the epithelium of the intestinal tract. When we set out we were not looking for the results we have seen. This is only a preliminary study; it is not completed, and we are presenting it because we thought it might be of interest to you. We have not killed any animals at the 1400 r. level which received marrow, so I do not know.

In response to the question whether other materials than bone marrow have been used, no, they have not been used as yet; we are just starting. However, I think the injection of lymphoid tissue of some type would be interesting. Since correlative studies regarding shielding of lymphoid tissue show earlier regeneration of both bone marrow and lymphoid tissue, and there is some evidence that bone marrow will provoke more rapid recovery of both bone marrow and lymphoid tissue, that will be done in the future. There are a lot of questions that have not been answered yet.

COMPARATIVE EFFECTS OF 400 KV. X-RAYS AND 19 MEV. (BETATRON) ELECTRON BEAM ON LYMPHOID TISSUES OF THE RAT. John B. Fuller (by invitation) and John S. Laughlin (by invitation), Chicago, Ill.

Abstract. The first purpose of these experiments was to learn the ratio of biologic activity of (1) a high energy (19 mev.) electron beam from the betatron, and (2) lower energy (400 kv.) x-rays from a conventional therapy machine. Another objective was to find out if any fundamental differences exist in the manifestations

of radiation damage in mammalian tissues, as reflected by gross and microscopic study, from 400 kv. x-rays and higher energy electron radiations.

Male albino rats, 28 to 29 days of age and weighing 65 to 75 gm., were employed. These were given *total body* irradiation in a single dose, under similar physical conditions, from the two sources of radiation under comparison. One hundred rats were given total body irradiation with the 400 kv. x-rays and the $LD_{50}/30$ days dose was 560 r. One hundred and twenty rats were given total body irradiation with 19 mev. electrons and the $LD_{50}/30$ days dose was 800 rep. There was a significant difference between these LD_{50} values. The 400 kv. energy x-rays were from 1.24 to 1.79 times more active in their lethal effects on rats of this age than were the 19 mev. electrons at a confidence level of 5 per cent. Smaller groups of rats were irradiated with the same doses of radiations of both types and sacrificed at intervals during the first 30 days post-radiation for comparative histologic and organ weight studies.

In the spleen and lymph nodes of rats treated with an LD_{50} dose of either high energy electrons or lower energy x-rays, the period of maximum destruction lasted for about 4 days post-radiation. From about 5 to 15 days post-radiation, extramedullary hemopoiesis in the red pulp of the spleen and in the medullary cords of the lymph nodes increased from minimum to maximum amounts in both groups. Lymphopoiesis in the splenic follicles and in the cortical reticulum of lymph nodes usually did not begin until about 10 to 15 days following LD_{50} doses of either high energy electrons or lower energy x-rays and often did not reach maximum amounts until after 30 days post-radiation. In contrast, in the thymus glands, with LD_{50} doses of either type of radiation, lymphopoiesis was well established by 7 to 10 days post-radiation.

In summary, the high energy electron beam is significantly less active quantitatively than the lower energy x-ray beam, and the magnitude of this difference is in keeping with the difference in specific ionization of the two. At the LD_{50} doses of each beam, however, the behavior of the treated animals is not significantly different during the first 30 days post-radiation, indicating that the high energy electrons and the lower energy x-rays act qualitatively by similar mechanisms on an organism as complex as the rat.

LESIONS OF THE ALIMENTARY TRACT OF DOGS EXPOSED TO WHOLE BODY X-RADIATION OF 300 TO 3,000 R. George Brecher and (by invitation) Eugene P. Cronkite, Bethesda, Md.

Abstract. This study forms part of a larger investigation directed toward the experimental evaluation of therapeutic procedures in radiation injury. Dogs were used because adequate blood samples can be removed daily and blood transfusions can be given readily. In order to establish the dose range within which therapeutic procedures could be evaluated, the mortality and survival time were determined at various dosage levels as well as the type and extent of damage produced and the capacity for spontaneous regeneration of injured tissues. Over 200 dogs were exposed to whole body irradiation of 300 to 3,000 r. using a 2,000 KVP industrial x-ray machine. To date none of the dogs exposed to doses of 400 r. or greater has survived. Two of 10 dogs died after exposure to 200 r., and 2 of 19 dogs after 300 r. The mean survival time of 62 dogs given 400 to 600 r. was 12½ days; of 12 dogs given 1,000 r., 6½ days. All of 12 dogs exposed to 2,000 and 3,000 r. died on the third or fourth day after irradiation.

Autopsies were performed on 150 irradiated and 12 control dogs. Dogs dying after doses between 300 and 1,000 r. almost invariably showed the picture of agranulocytic angina with ulceration, necrosis and massive bacterial invasion of the tonsils, and complete absence of granular leukocytes about the lesions. Ulcerations of this type were very rare elsewhere in the alimentary canal, but superficial erosions

overlying the site of mucosal hemorrhages were frequent in the stomach and intestines. After exposure to 600 r., slight damage to the crypt epithelium of the small intestine was evident in dogs killed on the first or second day, but this appeared completely repaired within 3 or 4 days. After 1,000 r., destruction of mucosal epithelium of the small gut was marked but regeneration of normal epithelium occurred regularly and was nearly complete by the fourth day. After exposure to 2,000 or 3,000 r., the lumen of the small gut contained a variable amount of bile-stained hemorrhagic fluid and frequently either the entire mucosa or longitudinal mucosal ridges were blood-stained. Microscopically, destruction of the epithelium was extensive. Nuclei of the remaining cells were much enlarged and vesicular in appearance; nucleoli were prominent. Bizarre mitotic figures were frequent. There was no evidence of normal epithelial regeneration through the fourth day, when all dogs had died, and probably none can be expected, since Martin and Rogers have shown that no regeneration of the mucosa occurs in loops of small intestine to which high doses of radiation were applied locally, even though individual dogs may remain alive for as long as 160 days after such local irradiation.

The present study indicates that deaths following doses of less than 1,000 r. were primarily due to the sequelae of pancytopenia, with the tonsils presumably serving as the portal of entry for bacterial invasion. In dogs exposed to 2,000 or 3,000 r., the functional disturbances produced by the extensive denudation of the mucosa of the small intestine were probably responsible for the extreme dehydration and shortened survival time in these animals. Since the survival time of other species of experimental animals is likewise shortened to 3 days after exposure to higher doses of radiation, and since similar but reversible lesions have been demonstrated by Tullis in mice irradiated with 1,100 r., it is suggested that doses of approximately 1,100 to 1,500 r. may represent the upper limit of the possible efficacy of supportive measures in the treatment of the syndrome of acute radiation injury. With greater doses the damage to the intestinal mucosa appears irreparable and of an extent incompatible with life.

Discussion

(Dr. Virgil H. Moon, Miami, Fla.) Some 10 years ago it was my privilege to present before this group the results of irradiation of approximately the same values as the higher values related in this paper. These experiments were made as another means for producing the syndrome of secondary shock. Several of Dr. Brecher's slides showed very similar conditions to those which we found in our animals which died 3 or 4 days after doses of from 2,000 to 3,000 r. I would like to ask whether other evidences of shock were present—whether there was capillary dilatation in the lungs and in other viscera. We saw capillary hemorrhages in the intestinal tract such as he demonstrated. It appears that we have been working in closely related fields, and I should like to hear further concerning the morphologic evidence of shock in the animals that received the higher doses.

(Dr. Brecher) Yes, we believe there is evidence of secondary shock. Dr. Laqueur has studied the hypophysis of these animals and has found what he feels is evidence of secondary shock in the form of degranulation of the basophils and other changes which he is now studying. Dilatation of the capillaries is quite variable both in the gut and in the lung.

(Dr. Moon) Was there hemoconcentration?

(Dr. Brecher) Yes, mostly on the last day of life.

MORPHOLOGIC CHANGES IN THE LYMPH: ESTIMATION OF PERMEABILITY AND LYMPHOPOIESIS. J. Furth and (by invitation) R. R. Bigelow, J. B. Kahn, M. M. Knoohuizen, and M. H. Ross, Oak Ridge, Tenn.

Abstract. With the aid of exteriorized lymph-venous anastomosis (Brown and Hardenberch), it is possible to study periodically the lymph of dogs without disturb-

ing the physiologic state of the animal. In the rat the lymph can be examined by the procedure of Bollman, Cain, and Grindlay. Such examinations of the lymph proved to be an excellent means of determining the state of lymphopoiesis in normal and x-rayed dogs and rats. The normal lymph of rats contains approximately 15,000 leukocytes per ml. About 4 to 6 per cent of these are large lymphocytes and the rest are small. Other types of white cells are rare. Erythrocytes are few. After exposure to LD_{50} doses of x-rays, the leukocyte count of the lymph drops to a few hundred within a few days and large lymphocytes become the predominating cell type. Some of these are in mitotic division and others have abnormal nuclei and vacuolated cytoplasm. Erythrocytes appear in increasing numbers, reaching counts averaging about 500,000 at 6 to 11 days after irradiation. The sinuses of lymph nodes become loaded with erythrocytes, which are engulfed by large mononuclears, and the hemoglobin is broken down to hemosiderin. The lymphatics become distended with erythrocytes and the rate of lymph flow rises. The erythrocyte count of the lymph is to some extent a measure of the degree of capillary damage. Extravasation of erythrocytes in tissue spaces and their diversion into the lymph compartment cause an apparent anemia. Destruction of extravasated erythrocytes and failure of their production cause a reduction of the total red cell mass (measured by radiophosphorus). These changes explain, at least in part, the anemia and shock of the acute hemorrhagic syndrome. Tables of cell and differential counts and photomicrographs were shown, correlating the blood and lymph picture with the anatomical changes invariably present in lymph nodes and lymphatics after massive irradiation.

Discussion

(Dr. George Brecher, Bethesda, Md.) I would like to ask Dr. Furth two questions. I believe the technic used in the dog was developed simultaneously by the Naval Medical Research Institute and by Dr. Furth. I understand there is some difficulty in having the lymph free of red blood cells, that one may occasionally get a back-flow of red cells into the cannula, and I wonder whether Dr. Furth will comment on that. The second question is whether a quantitative estimate can be made of the red cells lost in this fashion.

(Dr. Virgil H. Moon, Miami, Fla.) I should like to ask Dr. Furth some questions similar to those asked by Dr. Brecher. We have cannulated the thoracic duct in dogs when shock was developing or when it was present, and have noted the same change he illustrated so beautifully here: the high content of red blood cells in the lymph from the thoracic duct. This lymph would coagulate spontaneously in the tube, indicating a high degree of permeability of the capillaries in the areas drained by the thoracic duct. In these animals, during the early stages of shock, there was marked hemoconcentration. Was that noted in the early stages after irradiation? Furthermore, we observed in dogs and in human beings who survived the stage of acute shock following burns that there was a marked anemia, such as many others have observed.

(Dr. Furth) True, the technic was developed simultaneously in the Naval Research Laboratories by Brown and Hardenberch and their report is cited in our paper. However, there was no change in the lymph of their dogs, perhaps because they studied the first period and not the second period after irradiation, when the hemorrhagic syndrome sets in.

Dr. Brecher asked whether there was back-flow of red blood corpuscles. I believe there was not. A few erythrocytes are often observed in the lymph immediately after the establishment of the anastomosis. This soon clears up and there is no back-flow, as indicated by the white color of the lymph. At the Naval Laboratory the fistulas were occluded within about 3 days. This occurred also in our normal dogs but not in irradiated dogs in which the fistulas remained patent for 7 to 14 days, probably because radiation decreases the coagulation time.

Dr. Brecher also asked whether a quantitative estimation can be made of the fate of erythrocytes. This is being done. One can make a complete balance and measure the iron (erythrocyte) content in each organ separately. There is a tremendous increase of Fe^{59} in the spleen of irradiated dogs, somewhat less in the liver; the maximum increase of Fe^{59} per gm. weight of tissue occurs in the lymph node.

In response to Dr. Moon's question, we are very much interested to learn that similar changes occur in shock. As you recall, we described this change, but we did not say anything about its pathogenesis. Is the capillary damage or the shock syndrome primary? Or do both occur in the form of a vicious circle? This remains to be established.

RADIOSENSITIVITY OF THE IMMUNE RESPONSE. Frank J. Dixon, S. C. Bukantz (by invitation), David W. Talmage (by invitation) and Gustave J. Dammin, St. Louis, Mo.

Abstract. Investigation of the effects of x-radiation on antibody production has not only given us information which sheds light on the mechanism of antibody production, but also has suggested possible means by which the inhibitory effects of x-radiation on antibody formation can be counteracted. We will present three facets of the relationship between x-radiation and antibody formation.

First, in quantitating the radiosensitivity of the antibody response, we exposed groups of rabbits to 100, 200, 300, 500, and 700 r. of whole body x-radiation, and then measured their antibody response to I^{131} labelled bovine gamma globulin (I*–BGG) given 40 hours post-irradiation. One hundred and 200 r. caused only partial inhibition of antibody formation, while 300, 500, and 700 r. caused complete suppression in most animals.

Second, we determined the time necessary for x-radiation to suppress antibody production and the duration of this suppression. Several groups of rabbits were exposed to 500 r. x-radiation and each was given I*–BGG at a different time: 2 hours before, simultaneously with, 2 hours after, 7 hours after, 18 hours after, 40 hours after, 7 days after, and 40 days after x-radiation. Antibody response to I*–BGG given before or simultaneously with x-radiation was as large as the response in the absence of x-radiation. Antibody suppression by x-radiation increased in the rabbits given antigen 2, 7, and 18 hours post-radiation. Complete antibody suppression was seen in the 40 hour groups. Very slight recovery of the antibody-forming mechanism was noted in the 7 day group and some impairment persisted even in the 40 day rabbits.

Since the antibody response to an antigen given before or simultaneously with x-radiation was not affected by 500 r., which is sufficient to inhibit completely the response to antigen given 40 hours post-radiation, it would seem that only the initial phase of antibody production is radiosensitive. The radiosensitive material necessary for the initial phase of the antibody response progressively diminishes following x-radiation and is entirely gone in 40 hours. Once initiated, antibody production can be relatively radioresistant.

Third, we attempted to find whether this radioresistant antibody formation was specific, *i.e.*, would only form antibody to the specific antigen given before or with x-radiation, or whether once antibody production was underway, antibody could be made to a different antigen given subsequent to normally inhibiting doses of x-ray. We gave 500 r. to rabbits and immediately afterwards administered horse gamma globulin. Forty hours later we gave I*–BGG which elicited a subsequently demonstrable I*–BGG antibody response.

It appears that if the antibody-producing mechanism is active, it is relatively radioresistant, and if inactive, radiosensitive. Whether or not the preservation of the capacity to form antibody in the post-radiation period will prove of benefit to the

organism will have to be tested by relatively large survival studies. However, it appears that one of the injurious effects of x-radiation may be modified.

THE CHOLESTEROL OF HYALINE ARTERIOLOSCLEROSIS. Roger D. Baker and (by invitation) Eli Selikoff, Birmingham, Ala.

Abstract. The hyaline renal arterioles of 47 autopsied cases were observed in frozen sections treated with sudan IV and by the Schultz modification of the Liebermann-Burchard histochemical test for cholesterol. Forty-six of the 47 cases presented cardiac hypertrophy, and the majority were known to have had hypertension during life. The ages of the patients varied from 20 to 80 years. Sudanophilia of the hyaline arterioles was demonstrated in all 47 cases. The Liebermann-Burchard test was positive in the hyaline arterioles of 43 cases. In 4 cases it was impossible to identify arterioles. A control group of 20 cases, showing no hyaline renal arterioles in hematoxylin and eosin sections, was studied in similar fashion. In 16 cases the arterioles were not sudanophilic, in 4 cases they were. In 14 cases the arterioles gave a negative Liebermann-Burchard reaction, in 2 a positive reaction, and in 4 it was impossible to identify the arterioles.

In correlative histochemical study of various tissues of the body the following gave cholesterol-positive reactions: hyaline arterioles in other organs than kidney, atherosclerotic lesions of larger arteries, adrenal cortex, and myelin of the central nervous system. Sudanophilic substances which failed to give a positive Liebermann-Burchard reaction consisted of adipose tissue, sebaceous glands, "fatty change" of liver, lipochrome of cardiac muscle and of ganglion cells of spinal cord. The regularity of the positive Schultz modification of the Liebermann-Burchard reaction for cholesterol in hyaline and sudanophilic renal arterioles suggests that the process here is arteriolar atheromatosis, like that of the larger arteries.

Discussion

(Dr. Béla Halpert, Houston, Texas) I would like to ask whether Dr. Baker examined his frozen sections for isotropic and anisotropic substances. Cholesterol is isotropic and cholesterol ester is anisotropic. Perhaps some difference in the concentration of the lipid could have been obtained by this method of examination.

(Dr. Paul Klemperer, New York, N.Y.) Dr. Baker indicated in his remarks that he examined the hyaline material for substances other than lipid. I would like to know if he cares to mention what was the nature of these other substances and the relationship of the lipid, whether chemically bound or just adsorbed. I think it is important to emphasize that hyalin is not only lipid.

(Dr. Paul Gross, Pittsburgh, Pa.) I should like to ask whether Dr. Baker believes that the lipid demonstrated in the vessels may be responsible for, or have some relation to, sclerosis or fibrosis.

(Dr. Baker) Dr. Halpert, we did work with the polarizing light mechanism and found no double refraction in polarized light. My understanding is that the anisotropic effect is related to the occurrence of crystals present in the atheromas of large arteries, but not in hyaline arteriolosclerosis.

Dr. Klemperer, the McManus stain for glycogen is moderately positive. The hyalin of arteriosclerosis is not duplicated in atheromas usually. Since hyaline substances are often of protein nature, in hyaline arteriolosclerosis we may be dealing with a protein which is bound to lipid.

Dr. Gross, whether the hyaline substance is a precursor of fibrous tissue, I do not know.

THE ASSOCIATION OF ACUTE PANCREATITIS WITH ACUTE CORONARY THROMBOSIS. Caspar G. Burn, Brooklyn, N.Y.

Abstract. In our series of routine autopsies, of all ages, 10 per cent revealed evidence of acute pancreatitis. Further analysis showed that the highest incidence of

autopsies with acute pancreatitis was associated with heart disease, while cancers of all kinds, cirrhosis of the liver, and infection in general followed to a lesser degree. Of the autopsies showing pancreatitis in association with heart disease, in 30 per cent or more there were recent coronary thrombosis and infarction. Focal pancreatitis was found also without coronary occlusion in cases with hypertensive heart disease with moderate or severe coronary arteriosclerosis. There were 2 instances in which pancreatitis was found with inactive rheumatic heart disease. The pancreatitis consisted of focal ante-mortem necrosis of the acinar tissue and fat necrosis. The pancreatitis in these heart cases was minimal in degree. The possible significance of acute pancreatitis in relation to heart disease with special emphasis on coronary occlusion was discussed.

Discussion

(Dr. Jacob Werne, Jamaica, N.Y.) In my medicolegal jurisdiction I see many cases of coronary occlusion, and, if Dr. Burn's work is substantiated, we have been missing this coincident pancreatitis. I wonder whether some of these cases may not represent post-mortem autolysis of the pancreas, a very common finding in my experience. I would also be interested in knowing what proportion of these cases of pancreatitis were diagnosed on the basis of gross changes, such as flecks of yellowish softening indicative of fat necrosis, or whether there was actual disruption of tissue architecture by hemorrhage, as seen in hemorrhagic pancreatitis.

(Dr. Cornelia Hoch-Ligeti, Charlottesville, Va.) Next to cardiac lesions, the most pancreatitis was found associated with liver damage. What was the histologic picture of the liver in the cases of pancreatitis?

(Dr. Joseph F. A. McManus, Charlottesville, Va.) I was interested, as was Dr. Werne, in the time after death of these autopsies and the number of sections necessary to observe this lesion. A further question has probably been answered by Dr. Burn, that of subclinical pancreatitis. I wonder if there are any clinical features which would suggest anything of the sort.

(Dr. Burn) I was quite aware of what Dr. Werne has brought out, of course, and we were very careful to present material which was not due to post-mortem change. In fact, we eliminated a large amount of material that showed evidence of what we called post-mortem autolysis. These areas with special stains show very distinct changes that we feel are definitely ante-mortem in character. Although there may be a difference of opinion, these were considered to be ante-mortem lesions because many of them showed a definite cellular reaction of polynuclear leukocytes and a few lymphocytes as well as calcium increase. I do not think that these reactions can occur after death.

About not seeing the gross changes at autopsy, I think there is another difference that might be stressed. All of the autopsies showing these heart lesions were on people over 50 years of age, while some were of even an older age group. Most of the cases Dr. Werne sees are probably of younger age, and I have no data bearing on that particular group. Another factor which has to be considered is that I notice when residents and younger men do autopsies they miss these lesions very readily. One has to look for them with great care and dissect the pancreas carefully to demonstrate these gross changes.

With regard to the liver changes, we have made incomplete studies so far, but in most of the livers chronic passive congestion was found. Some livers showed fatty change, but little else was noted.

Most of the autopsies that were included in this series were done less than 8 hours after death, while only a few were performed 24 hours after death.

The number of routine sections was a real limitation in this study. As routine sections for any autopsy usually include one or two sections of pancreas only, we recently have been making multiple sections of the pancreas from these cases, and have demonstrated lesions that were not found by a single routine autopsy section.

I think the incidence of these lesions would be higher under this serial method of examination.

In regard to the clinical manifestations, I know of one or two instances with clinically demonstrated coronary occlusion in which the amylase studies were made. Some of these had very high amylase values for the blood serum. What the significance of that is, I do not know.

THE PULMONARY VASCULAR BED IN CHRONIC HEART FAILURE. Alvin J. Cox, Jr., and (by invitation) John S. Cheredes, and Watson M. Lacy, San Francisco, Calif.

Abstract. The maximum capacity of the pulmonary vascular bed has been estimated by perfusion of human lungs post mortem through the pulmonary artery with kerosene. Eighty cases of various types were studied. Four cases of chronic pulmonary disease associated with clinical cor pulmonale presented pronounced restriction of perfusibility of the pulmonary artery to as little as one-tenth of the maximum for control lungs. Lungs from 23 patients with chronic heart failure resulting from coronary arteriosclerosis, arterial hypertension, and rheumatic valvular disease also exhibited markedly restricted perfusibility. A relationship between resistance of the lungs to perfusion and weight of the right ventricle has been demonstrated. Although the bronchial arteries in some of the specimens were enlarged, the degree of obstruction to pulmonary arterial perfusion in the abnormal lungs suggests that this was the most important alteration in the pulmonary vascular bed.

STUDIES ON THE PERMEABILITY OF THE ENDOTHELIUM TO LIPIDS IN RELATION TO ATHEROSCLEROSIS. Aaron Kellner and (by invitation) D. C. Dju Chang, New York, N.Y.

Abstract. The lipids of the circulating blood have long been implicated in the pathogenesis of atherosclerosis, and during recent years particular emphasis has been placed upon the blood cholesterol, phospholipids, lipoproteins, and chylomicrons in relation to this process. The atheromatous plaque, however, is morphologically outside the blood stream, and the lipids that precipitate within the arterial intima during the development of atherosclerosis are lipids that have crossed an endothelial membrane and passed out of the blood stream to enter the tissue spaces of the artery wall. It becomes important in this connection, therefore, to know more fully the lipid content of the tissue fluid and the factors that govern the permeability of the endothelium to lipids.

A technic has been devised for cannulating the subcutaneous lymphatics of the lower leg of rabbits whereby it has been possible to obtain quite regularly 0.5 to 2.5 cc. of tissue lymph; the composition of such tissue lymph is, according to Drinker, practically identical with that of the extracellular or tissue fluid. Tissue lymph and blood serum were obtained from normal rabbits and from rabbits with hyperlipemia induced by cholesterol feeding or by the injection of alloxan or surface-active agents. In normal animals it was found consistently that the level of each of the major lipid fractions (cholesterol, phospholipid, and neutral fat) of the tissue lymph was only one-quarter to one-half that of the corresponding lipid in the blood serum. The glucose content of the tissue lymph, by comparison, was usually 80 per cent or more of that of the blood serum. These observations indicate that the capillary endothelium is normally permeable to lipid particles but only to a limited degree.

Rabbits fed cholesterol were found to have a significant increase in the quantity of lipid in the tissue lymph, and it is noteworthy that the disparity between cholesterol and phospholipid previously reported in cholesterol-fed rabbits, a high cholesterol and a relatively low phospholipid, was present also in the tissue lymph. In the hyperlipemia associated with alloxan diabetes, on the other hand, it was found that despite an enormous increase in blood lipids, the lipid content of the tissue lymph remained virtually unchanged. This finding suggests that either the lipid

particles in the blood were of such a nature that they could not pass the endothelium, or that alloxan diabetes in some manner altered the permeability of the endothelial membrane. This observation is of considerable interest in view of the studies by Duff indicating that alloxan diabetes protected cholesterol-fed rabbits against the development of experimental atherosclerosis. Finally, we have made the observation repeatedly that lymph obtained from rabbits with grossly milky serum was water-clear in almost every instance, indicating that very large lipid particles (chylomicrons) do not readily cross the endothelium. This finding casts considerable doubt on the hypothesis that chylomicrons may play an important rôle in the pathogenesis of atherosclerosis.

Discussion

(Dr. Jacob Furth, Oak Ridge, Tenn.) In studies of lymph permeability the time factor is important. When we introduce a certain substance in the blood, there will be a gradual drop of it in the blood and gain in lymph until an equilibrium is reached between blood and lymph. To express the permeability grade, one has to know the equilibration slope and time in addition to the activity of the lymph.

(Dr. Paul Klemperer, New York, N.Y.) I wonder if this permeability depends only on the endothelium, or are there not other structures in the capillaries which must be considered. Have studies been done on the difference in permeability for lipids after cortisone and desoxycortisone, which seem to change the permeability of capillaries?

(Dr. O. J. Pollak, Quincy, Mass.) I think the assumption that the endothelium in atherosclerosis is intact is erroneous. I am going to show in my coming paper that the endothelium is damaged, and that, of course, would make all the difference.

(Dr. G. Lyman Duff, Montreal, Que.) I think Dr. Kellner's observations are most interesting and suggestive. What he is measuring is clearly the permeability of capillary endothelium. I want to ask whether he has any evidence, beyond presumption, that the permeability of the endothelium of arteries is comparable with that of the capillaries.

(Dr. Herbert C. Stoerk, Rahway, N.J.) I would like to ask Dr. Kellner whether he has considered the possibility that the clearing of lipid from the plasma may be due to the activity of cells belonging to the reticulo-endothelial system. I am asking this because we have had occasion to autopsy rats from experiments by Dr. Robinson which were injected with rather large amounts of a detergent. These animals had very marked lipemia and showed changes greatly reminiscent of those seen in diseases of thesaurismosis (Gaucher's, Niemann-Pick's disease). The cytoplasm of tissue-histiocytes and of monocytes in these animals was engorged with lipoid material.

(Dr. Kellner) The time for equilibration was not a factor in our experiments because the lipemia that is produced following the injection of alloxan or surface-active agents is a lipemia that lasts for hours and days, and there is ample time for equilibrium to be reached.

In reply to Dr. Klemperer's question, there may well be other structures in the capillary wall, various cement substances, for example, which play an important rôle in permeability. There may be a great many factors in the lipids themselves which determine permeability; molecular size, shape, electric charge, relationship to protein, and perhaps other factors. We have no data as yet on the effect of cortisone and ACTH on permeability; these experiments are in progress at the present time.

Dr. Duff's comment about capillary endothelium is well taken. The observations we have made are limited to capillary endothelium and need not necessarily apply to the endothelium of the aorta or the coronary arteries. We have studied the permeability of capillary endothelium first because that was most feasible from a technical point of view. It would certainly be desirable to cannulate the lymphatics

of the arch of the aorta and examine the lipids in such lymph. However, technical difficulties make that a most difficult procedure at the present time.

It is unlikely that histiocytes are responsible for the differences observed between serum lipid and the lipid in the tissue lymph. We have not observed histiocytes in the area of cannulization, and we have not observed lipid-filled histiocytes in the lymph. The tissue lymph we have obtained contains a few red cells and virtually no white blood cells.

THE RELATIONSHIP OF AGEING CHANGES TO THE DEVELOPMENT OF ARTERIOSCLEROSIS IN THE HUMAN AORTA. Maurice Lev and (by invitation) Catherine M. Sullivan, Chicago, Ill.

Abstract. The aortas of 57 patients with no evidence of hypertension, diabetes, or hypercholesterolemia were studied grossly and histologically. Methods of fixation and staining were adapted to the special study of muscle, elastic tissue, collagen, intercellular substance, and basement membranes.

The aorta grossly during fetal life and at birth presents whitish streaks located in the upper margins of the sinuses of Valsalva, at the exit of the bracheocephalic vessels, and at the point of entry of the ductus arteriosus. Within the first several years of life, there is a further appearance of irregular linear criss-cross formations at the exit of the intercostal vessels, celiac axis, and superior and inferior mesenteric arteries. Between 5 and 10 years of age, and rapidly thereafter, the lowermost segment of the aorta becomes more severely involved, while the ascending aorta shows relatively little change.

Beginning at about 3 years of age, yellow streaking (fat) is noted in the plaques in the regions of the sinuses of Valsalva. By the age of 20 to 30 years this streaking has involved the transverse and descending aorta, following closely the original white streaking observed. After the age of 40 the distribution of fat becomes quite diffuse, but retains the above stated predilection from the standpoint of severity.

Histologic examination of the white plaques seen from birth reveals a proliferation and re-orientation of elements of the intima with concomitant changes in the adjacent media. Such changes are present to a lesser degree in other parts of the aorta. The intimal change consists of a proliferation, in the direction of blood flow, of elastic, muscle, and collagenous fibers with an increase in ground substance. The adjacent media shows (1) a thickening and widening of the elastic nets, with accentuation of their longitudinal component, (2) hypertrophy of the muscle cells, and (3) increase in ground substance. This reaction in the lowermost portion of the aorta shows greater muscular hypertrophy and lesser elastic change. This process may be called endarteriohypertrophy, and is similar to the process of endophlebohypertrophy described by Lev and Saphir.

Beginning in fetal life and progressing with age, degenerative changes occur in plaques of endarteriohypertrophy. These consist of (1) dissolution of elastic fibers, (2) degeneration of muscle cells, (3) increased and decreased staining of the ground substance, (4) increased and decreased staining of the reticular and glycoprotein components of the basement membranes around elastic and muscle tissue, (5) appearance of degenerated cell forms in the intima in addition to inflammatory cells, (6) increase in collagen fibers with the production of fibrotic and calcific zones, and (7) the appearance of vasa vasorum in media and even in the intima. This process may be called endarteriosclerosis.

In some spots of endarteriosclerosis fat is deposited in the ground substance, muscle fibers, and on elastic fibers. With progressing age these fat accumulations are larger, forming elevated zones. Secondarily, macrophages appear, and later a marked increase in collagen fibers ensues. This process may be called endarteriosclerosis with

atheromatosis. Independent of the above process, calcification occurs in the media at about 40 years of age, and progressively thereafter. This may be called senile arteriosclerosis.

Thus it may be postulated that endarteriosclerosis is related to hydrodynamic stress, specifically, to tangential forces and lateral pressure, leading to endarteriohypertrophy and secondary degenerative changes—endarteriosclerosis. The latter is the nidus for atheromatosis with its still further secondary degenerative changes.

Discussion

(Dr. D. Murray Angevine, Madison, Wis.) I would like to ask Dr. Lev whether he has any evidence to indicate that there is continuity in this process in so far as age is concerned.

(Dr. Cornelia Hoch-Ligeti, Charlottesville, Va.) May I ask what was the cause of death in the 3-year-old child from whom the aorta was shown?

(Dr. John T. Prior, Syracuse, N.Y.) I would like to ask whether the aortas, particularly those of infants between 0 and 1 year of age, have been serially sectioned, and whether there has been any attempt to show that there is a greater distribution of these changes as one progresses caudally in the aortas? We have been doing this for some time and have had a large number from the newborn sectioned serially. We have not studied the gestation period; we have only stillborns. We think there is a greater incidence of these changes, which Dr. Lev has described, proximally rather than distally, as might be expected. Also I would like to know whether there is any sex difference; whether it is more severe in the male than the female, analogous to the work Dock described in coronary arteries?

(Dr. Jesse L. Carr, San Francisco, Calif.) I would like to know about the health and general status of the mothers of these infants and if anything is known about their lipid metabolism.

(Dr. Lev) As concerns Dr. Angevine's question, the purpose of our work was to see if there was a general trend in changes with advancing age—and we feel that such a trend exists—hence there is continuity. There is one point which I did not have time to discuss which should be added: In my description I stated that the deposition of fat was more or less related to the areas of maximal endarteriohypertrophy and sclerosis. This is a focal process. There is, however, a general process going on with advancing age. This generalized change concerns itself with hypertrophy of all the elastic fibers and reaches its maximum at about 20. After 20 no more hypertrophy of elastic fibers occurs, but now degenerative changes set in in the elastic tissue, with the deposition of calcium, as described by others. This may have a secondary effect on the deposition of fat, but it has no direct effect because lipid changes occur from the age of 3 years on. The tendency toward large accumulations, however, is seen after 30 years. Here, then, very likely other factors creep in, concerned with the deposition of lipids, which have been described by Dr. Kellner and others. Thus, in answer to Dr. Angevine, I believe there is continuity of the changes I have described, provided one leaves room for the other factors.

Concerning serial sections: We have not done serial sections on the newborn aortas. In all our cases, young and old, we have sectioned five areas: the ascending aorta; the aorta at the exit of the bracheocephalic vessels; below the entry of the ductus arteriosus; above the exit of the celiac axis; and the most distal portion of the aorta. I think if we had done serial sections, we would have found finer changes, but I do not think they would have altered the general trend. It is true that the hypertrophic changes that I have described are not limited to the formations seen grossly, but they are maximal in these spots and less marked elsewhere. I think that we are dealing here with a surface reaction related to friction which operates over

the entire inner surface of the aorta but which is maximal at points of maximal mechanical stress. We have seen the same process in the veins, where it is much easier to study and interpret.

I do not know anything about sex differences. Sixty-five cases are insufficient for statistical analysis. When we have 300 to 400 cases, we will go into that.

Concerning the state of the mothers of the newborn or fetuses studied: It was not unusual; we did not, however, investigate the question of hypercholesterolemia in the mother. All of the aortas studied after birth were from patients without hypercholesterolemia, diabetes, or hypertension.

I do not remember the cause of death in the 3-year-old with lipid deposits in the aorta. This is not an isolated finding; it is seen very commonly at 3, and perhaps earlier.

INTIMAL ALTERATIONS IN ALARM REACTION. AN ETIOLOGIC FACTOR IN ATHEROSCLEROSIS. O. J. Pollak, Quincy, Mass.

Abstract. Blood vessels of persons who have experienced shock show hydropic vacuolization of intimal endothelial cells which is comparable to alterations induced by intravascular injection of macromolecular matter into rabbits. Numerous cushion-like subintimal foam cell aggregates which are grossly visible as early yellow atherosclerotic plaques are found in young individuals, children, and infants who suffered shock. Hydropic swelling of vascular endothelial cells as part of the alarm reaction facilitates intimal-subintimal cholesterol deposition. The phenomenon might well represent an etiologic factor in the development of atherosclerosis as neither hypercholesterolemia nor increase in the number of S_f 10-20 molecules of lipobetaglobulin alone suffice to initiate atherosclerosis. Hydropic swelling of intimal cells apparently results from the change in osmotic pressure caused by hypo-albuminemia of shock. Certain local factors influence the localization of the alterations. The phenomenon aids in the explanation of the episodic character of atherosclerosis and of the progress of the disease with advancing age.

Discussion

(Dr. Maurice Lev, Chicago, Ill.) I would like to ask if Dr. Pollak feels that human material can be studied from the standpoint of endothelium after autopsy.

(Dr. Pollak) That is a very pertinent question, and we have gone into this very carefully. The only defense I can make is in our controls. Over 100 routine appendices which were not perforated were free of vascular alterations. The same is true in the rabbit materials. I can safely say I have examined 2,000 rabbits. I have not seen these changes unless animals were exposed to stress, either to intravenous injections of foreign materials or to electric shock. I have injected some animals with cortisone and I have found the same changes in them. I have not yet tried other adrenal hormones.

THE REACTION OF THE PULMONARY ARTERY TO MINUTE EMBOLI. W. B. Wartman and (by invitation) R. B. Jennings, Chicago, Ill.

Abstract. Rabbits were given injections into the ear veins of saline suspensions of minute fibers of filter paper and of mixtures of filter paper and rabbit fibrin or rabbit whole blood. The emboli either became impacted in the pulmonary arteries or adhered to the intima. In one rabbit an embolus adhered to the endocardium of the right ventricle. An acute inflammation resulted which subsided after about 1 week. Organization of the embolus and inflamed arterial wall resulted in retraction of the embolus and diffuse or eccentric scarring of the intima. Vascularization of some of the scars was observed, but hemorrhage and deposition of lipoids were not seen. Intravascular bridges occasionally formed.

The filter paper fibers were soon surrounded by a foreign body granuloma and became localized in either the intima, media, or adventitia. They frequently passed

entirely through the wall of the affected artery, causing varying amounts of injury, and were found in the adventitia or perivascular lung tissue. This is interpreted as indicating the existence of a mechanism for ridding the circulation of foreign material in the blood. Localization of the fibers in lymphatics situated between the bronchi and arteries is described.

Injection of minute emboli of clots of human fibrin having an average diameter of 0.1 mm. did not cause similar lesions. This failure, which is at variance with previously reported work, may be explained by the use of extremely small emboli and a different experimental technic.

Discussion

(Dr. Joseph A. Cunningham, Birmingham, Ala.) I would like to ask if Dr. Wartman found any evidence of transfer of these cotton fibers to the liver or spleen, and whether in any of the granulomas he found stellate inclusions.

(Dr. Wartman) We did not find fibers in either organ, and we did not find stellate inclusions.

MORPHOLOGY OF THE VASCULAR LESIONS IN "THROMBOTIC THROMBOCYTOPENIC PURPURA" WITH DEMONSTRATION OF ANEURYSMS.* J. L. Orbison (by invitation), Cleveland, Ohio.

Abstract. The disease entity known as "thrombotic thrombocytopenic purpura" or "thrombocytic acroangiothrombosis" is characterized clinically by thrombocytopenic purpura, hemolytic anemia, and neurologic manifestations. The outstanding histologic manifestation is occlusion of the small arteries, arterioles, and arteriolar-capillary junctions by amorphous or granular acidophilic material considered to be platelet thrombi. These lesions have been present in every organ of the body except the skeletal muscle, but have been seen only rarely in the lungs. That the thrombi are composed, at least in part, of platelets seems to be the best assumption, but it should be pointed out that direct proof is not available.

Some investigators have postulated that no primary vascular lesion is present to predispose to the occlusions. However, the material examined here revealed proliferation of endothelium in the absence of thrombi, and fibrinoid changes in the vessel walls with partial destruction of the elastic lamina. These observations are in accord with those who believe that a primary vascular lesion does exist and predisposes to the occlusions.

A histologic manifestation that has been previously reported but not previously studied are the prominent vascular dilatations associated with the occlusions. These were seen in both of the cases studied, and although they occurred only occasionally in one case, they were very prominent in the other. These changes were most obvious in the heart but occurred also in brain, pancreas, and kidney. A reconstruction of vessels in the heart revealed these aneurysms to occur occasionally in small arteries and arterioles, but the principal site was at the arteriolar-capillary junctions. The aneurysms of arteries and arterioles frequently showed focal or complete loss of the elastic lamina and those at the arteriolar-capillary junction had a wall composed only of a single layer of endothelium. The lumina of the aneurysms were almost completely filled with either thrombi or proliferated endothelium, or a combination of the two.

The presence of such aneurysms adds to the evidence in favor of a primary vascular lesion since aneurysms are commonly the result of degenerative and destructive lesions of the vessels and do not usually occur as a result of a bland thrombus. Further significance is added to this evidence by the recent observation that the hemorrhages in purpura, apparently the result of a vascular injury, also occur at the arteriolar-capillary junction.

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

Discussion

(Dr. Paul Klemperer, New York, N.Y.) I am glad I listened to this paper because up to now I was never quite convinced of a primary vascular alteration as a basis for this peculiar disease. I was always struck by the peculiar dilatation of exactly that site which Dr. Orbison has shown, and I think this has now been quite definitely demonstrated by the serial sections and reconstruction of the actual aneurysm formation. We could see that that dilated part of the artery is markedly thin. One point I wonder about is the question of the origin of the hemorrhage in these cases. I have paid much attention to examining that and my conclusions, incorrect probably, were that the actual blood extravasation occurs in the venous portion of the vascular tree and not in the arteriocapillary junction. I thought the main reason for this was that the pressure from behind or proximal to the veins is so diminished that blood in the veins accumulates, stasis occurs, and blood escapes.

(Dr. Orbison) I want to thank Dr. Klemperer for his remarks. It has been reported several times that venous dilatation, venous occlusion, and venous hemorrhage occur in this disease. In this reconstruction we were unable to identify any veins or venules involved in the process. The only hemorrhage which we identified in this particular reconstruction occurred around the aneurysm of a small arteriole. In the section in which hemorrhage occurred the whole vessel wall was amorphous, bright-pink staining, and looked very much like a fibrinoid reaction. I believe that venous and perivenous hemorrhage has been considered as a feature in this disease because of the marked aneurysmal dilatation which we see in some of these arterioles with extreme thinning of the walls. I am unable to identify whether I am looking at an arteriole or a venule until I follow their relations in serial sections.

POST-MORTEM PULMONARY EDEMA. Stanley H. Durlacher and (by invitation) William G. Banfield, Jr., and A. Dorothy Bergner, Baltimore, Md.

Abstract. The ratio of lung to body weight has been determined in rabbits immediately, and at intervals, after sacrifice by a variety of procedures. Methods of sacrifice have included exsanguination, air embolism, electrocution, ether, rabbit punch, pentobarbital sodium by various routes, and intracardiac saturated magnesium sulfate solutions. Histologic examination of the lungs was made in all instances. The ratio of lung weight to body weight increased after death by all methods except exsanguination. The greatest increase occurred in animals sacrificed with pentobarbital sodium or magnesium sulfate solutions when introduced into the blood stream rapidly and in such fashion that high concentrations were present in the pulmonary vasculature after death. Under these conditions the lung weight to body weight ratio was two or three times greater after a 3-hour interval than was the ratio found in animals killed by the same materials administered by other routes. Increase in lung weight was due largely to accumulation of proteinaceous fluid within the alveoli, although a small portion of the increased weight was due to clotted blood remaining in the pulmonary blood vessels. When quantitative estimations of the degree of pulmonary edema present at autopsy are desired, rapid exsanguination is the most satisfactory method of sacrifice. In instances in which this procedure is not feasible, air embolism should be used.

Discussion

(Dr. Jacob Werne, Jamaica, N.Y.) I would like to emphasize the last statement of Dr. Durlacher by pointing out that in my experience with human material there is no proof that pulmonary edema can arise post mortem. As an illustration I cite the case of a newborn who died as the result of a skull fracture and was then thrown into an incinerator. Autopsy 1 day later showed extremely pale lungs, free of congestion or edema. In any routine medicolegal practice one has an opportunity to perform autopsies at varying intervals of time on subjects dying immediately fol-

lowing violence. Under such circumstances when death occurs rapidly, particularly in young subjects, it is the rule to find lungs free of congestion and edema, regardless of the post-mortem interval. When prolonged shock precedes death, or when death agony is prolonged, congestion and edema are commonly encountered. Their presence is significant at times in understanding the cause and mechanism of death. I believe it may very well be that the animals which are shown to have developed "post-mortem edema" may not have been dead at the time they were so considered. We know that "vital" reactions continue after clinical death and we also know that the exact time of occurrence of clinical death, particularly in animals, is difficult of determination. It is significant that in the experience reported by Dr. Durlacher and his associates, some types of death, such as electrocution, were unassociated with the development of this edema. I consider that this is further evidence that the development of this edema in certain types of "death" may actually be an ante-mortem rather than a post-mortem phenomenon. The profound pulmonary congestion and edema seen in human beings dying of barbiturate poisoning are noteworthy.

(Dr. Paul Gross, Pittsburgh, Pa.) I should like to ask Dr. Durlacher if there is any relationship of the development of edema to the coagulability of the blood post mortem.

(Dr. Durlacher) When pushed by Dr. Werne I will comment also that I have analyzed a small series of infants, that we are studying thoroughly, who died from unknown or minor disease or pulmonary inflammation. We have not been able to confirm the observations made in rabbits in this human material.

Of course, the question of the coagulability of the blood comes up. We showed how much dilatation of the vascular bed of the lungs was reflected in the lung weight. In the exsanguinated group we removed enough blood to reduce the post-mortem blood pressure. All other groups had a slight but significant post-mortem blood pressure of a few mm. of water. In the absence of this pressure, edema did not form. In the rabbit punch group as opposed to our electrocution group, there was a significant quantitative difference in the amount of edema formed, and the only thing which correlated with the lung weights was the degree of pulmonary edema observed by microscopic examination, so I feel pretty confident that we are actually measuring significant differences in alveolar edema, and not whether the blood had clotted in the vessels.

THE SPLEEN AND HYPERSPLENISM. R. G. Leffler (by invitation), Bethesda, Md.

Abstract. Eighty-seven spleens of various states of hypersplenism and 214 control spleens were examined histologically with special reference to the marginal zones of the malpighian follicles. Prominent widened marginal zones composed of "lymphoblasts" were found in all but one of the examples of hypersplenism, and this feature was absent in all but 14 of the control spleens. The aberrant case of hypersplenism had been treated with x-ray to the spleen and the histologic picture was largely obscured by fibrosis. The 14 control spleens included 3 cases of amyloidosis, 8 ruptured spleens, and 3 cases of cardiopulmonary disease. The relationship of the spleen to hypersplenism and the usefulness of the prominent marginal zones as a constant feature in the histopathologic diagnosis of hypersplenism were discussed.

Discussion

(Dr. Oscar B. Hunter, Jr., Washington, D.C.) I did not hear any reference to myeloid metaplasia and I would be interested if Dr. Leffler could make reference to the incidence of myeloid metaplasia in the spleens he examined. I would also be interested to know whether he has had the opportunity to study any hypersplenic tissue or spleens taken from patients suffering from malignant disease, such as leukemia.

(Dr. G. A. Nedzel, Bethesda, Md.) Were any reticulum stains done to determine

whether a ring of reticular fibers is present between the compact zone of small lymphocytes and the outer zone of large lymphocytes?

(Dr. Béla Halpert, Houston, Texas) Were there accessory spleens present in any of these cases, and if so, did they show the same change as the spleen proper?

(Dr. Jesse L. Carr, San Francisco, Calif.) What is the thymus gland like in these cases?

(Dr. Leffler) With regard to the question about myeloid metaplasia, I did see that in a number of spleens of hypersplenism and in a number of spleens of the control series. I was not able to make any correlation concerning the degree of myeloid metaplasia with the type of hypersplenism, nor were there any areas where it seemed to me myeloid metaplasia was most severe.

Concerning malignant disease of the spleen: there were a number of cases in which the disease was terminal, and in these spleens very often there were enlargement and pale-staining characteristics of all of the lymphocytes of the malpighian follicles.

Concerning the special stains, I did a few reticulum stains in the control cases and on the spleens from hypersplenism, and I saw a suggestion of a line of fibers separating the mantle zone from the marginal zone.

There were accessory spleens in a number of these cases of hypersplenism and in all cases they showed exactly the same features as the major spleen.

I did not study the thymus gland particularly, and I am not aware of any special change in the thymus gland in hypersplenism.

EXPERIMENTAL ENDOGENOUS LIPOID PNEUMONIA. Paul Gross and (by invitation) J. H. U. Brown and Theodore F. Hatch, Pittsburgh, Pa.

Abstract. Exposure of a group of 50 rats to finely divided Sb_2O_3 (mean diameter of 0.6 μ) in an inhalation chamber for periods up to 14 months produced a chronic lipoid pneumonia. The latter is characterized by much sudanophilic material and needle-shaped crystals within the alveolar spaces. While some of the lipoid is found within swollen macrophages, much of it also is found within the cellular débris which fills many alveoli. Considerable fibrous thickening of alveolar walls is present as well as focal fibrosis about fusiform clefts (crystal spaces). There is a general increase in the density and complexity of the reticulum pattern of the lung.

An exogenous origin of the alveolar lipoid is precluded by the experimental procedure employed. The dust for inhalation was prepared by atomizing an aqueous suspension of sized Sb_2O_3 and drying the mist while it traversed a heated tube prior to passage into the inhalation chamber. Failure to find similar lesions in two comparable control groups (50 rats each) excludes experimental factors other than Sb_2O_3 as causative of the lipoid pneumonia. Still another group of rats served not only to offer additional evidence that the intra-alveolar lipoid deposits are due to Sb_2O_3 but also to show that these lesions are not dependent upon the elaborate inhalation setup. These animals were given a single intratracheal injection of Sb_2O_3 as an aqueous suspension with the result that intra-alveolar lipoid deposits with perifocal fibrosis were noted as early as 2 months later. Intratracheal injections of other materials: clay, Fe_2O_3, and Al_2O_3, in comparable amounts and particle size, failed to produce such lesions. It is probable that the cytoplasmic inclusions of finely divided Sb_2O_3 so derange the metabolism of the alveolar macrophage as to lead to intracellular accumulations of lipoids with ultimate cell death and rupture. This concept is similar to the hypothesis suggested by Fallon for the pathogenesis of silicosis. Fallon found increasing amounts of extractable phospholipids in rabbit lungs with increasingly severe silicotic involvement.

Discussion

(Dr. Tracy B. Mallory, Boston, Mass.) This lesion which Dr. Gross has described and illustrated for us is closely similar to a lesion which is not infrequently seen in man. Probably most of you who see very much resected pulmonary tissue are aware

that behind a bronchial obstruction the alveoli commonly become filled with lipid-containing macrophages which give the histologic reactions of cholesterol and show a very high percentage of cholesterol on chemical analysis. Occasionally we have seen the same process in lungs in which no bronchial obstruction could be demonstrated. The x-ray and clinical picture often closely simulated bronchogenic carcinoma so that extensive resections of lung tissue have frequently been done for this completely benign lesion. I feel sure there must be members of the audience who have seen similar lesions. I should like to ask Dr. Gross if he feels that there is any evidence that the antimony trioxide used in these experiments may have caused bronchial obstruction by cicatrix formation or prolonged and persistent bronchial spasm.

(Dr. Gross) I am glad, Dr. Mallory, that you brought up one of the clinical implications of this experimental work. The pneumonia of cholesterol type to which Dr. Mallory has just referred was described by Robbins and Sniffen, from material at the Massachusetts General Hospital. In these cases the involvement was limited to one lobe or one or more segments of one lobe whereas in the experimental disease all portions of both lungs are involved. Contrary to the findings in the above surgical specimens no association of the experimental pneumonia with bronchial obstruction was noted. While spontaneous purulent bronchiectasis with perifocal pneumonia was common in the control animals, few instances of bronchial disease were found in the experimental group. Some of us have autopsied cases of lipoid pneumonia when no history of exogenous lipoid could be obtained but an exogenous origin was nevertheless assumed because of the prevailing opinion. I believe the possibility of an endogenous origin should also be entertained, at least in those cases in which a history of exogenous origin cannot be obtained. Another implication of this experimental work bears on pneumoconiosis. Fallon emphasized the similarity of the silicotic nodule to the tubercle. He pointed out that while the tubercle is a tissue response to the lipoid of the specific bacterium, the silicotic nodule is a similar response to phospholipids. The phospholipid content of silicotic rabbit lungs was found to increase with the severity of the pneumoconiosis. This was thought to indicate alterations in cellular metabolism induced by the silica which lead to accumulation of lipoids. The latter caused fibrosis. This concept is similar to the one expressed in our paper. Some additional support was recently obtained from a study of a case of pneumoconiosis due to bauxite fumes (Shaver's disease). Under polarized light innumerable large and small refractile lipoid crystals are visible in frozen sections where abundant sudanophilic material is also demonstrable. No indication of these findings is noted in the routine hematoxylin and eosin sections.

COEXISTENT GASTRODUODENAL AND CEREBRAL LESIONS IN INFANCY AND CHILDHOOD. Hans G. Schlumberger, Columbus, Ohio.

Abstract. In a series of 246 consecutive autopsies on infants and children I found 8 cases of acute duodenal ulcer and 2 of gastromalacia. All were associated with a cerebral lesion. The youngest was a 6-day-old infant, the eldest a boy of 14 years. The duodenal ulcers were single in 6 instances and multiple in 2. They were proximal to the ampulla, usually within a few mm. of the pylorus. None had perforated into the peritoneal cavity, but 3 had eroded the pancreas and 3 others had penetrated the layers of smooth muscle. In 3 cases the ulcer had been the source of massive intestinal hemorrhage. The 2 cases of gastromalacia had perforated before death, as manifested by the presence of hemoperitoneum and peritonitis at autopsy. In one there was also ulceration and perforation of the esophagus just above the level of the diaphragm. The duodenal as well as the gastric lesions showed only a scanty infiltration with inflammatory cells. Both were associated with microscopic areas of necrosis and erosion in the neighboring mucosa. In these regions thrombi sometimes were present in the small vessels of the submucosa. The ulcers probably origi-

nated as similar superficial lesions in the mucosa which sloughed and exposed the underlying tissue to the digestive action of the gastric juice, producing the grossly visible crater.

The cerebral lesions found associated with these changes in the stomach and duodenum were as follows: tuberculous meningo-encephalitis, 3 cases; bulbar poliomyelitis, 2; and intraventricular hemorrhage, sinus thrombosis with cerebral hemorrhage, severe anoxia, edema, and microcephaly, each 1. None of the lesions was limited to the hypothalamus; however, this region and the rest of the brain stem were always involved. The effect that injury to these areas has on gastric secretion and motility through increased or decreased activity of the autonomic nervous system is well known and has often been linked with the formation of acute gastroduodenal ulcers. That a humoral mechanism may also play a rôle is indicated by Selye who stated that exposure to stress in the presence of lesions in the pituitary and adrenal glands may lead to an inadequate response of these organs, one manifestation of which is the occurrence of gastro-intestinal erosions. Lesions of the hypothalamus may have a similar effect, for recently Hume and Wittenstein have shown that the intact hypothalamus is essential for the normal release of pituitary adrenocorticotropic hormone and adrenal corticoids in response to stress.

Discussion

(Dr. Peter Gruenwald, Brooklyn, N.Y.) Ulcerations in the esophagus appear quite frequently in infants both with and without brain lesions, in fact, much more frequently in the esophagus than in the stomach or duodenum. I should like to know whether Dr. Schlumberger found more cases of esophageal ulceration than the one demonstrated here.

(Dr. John H. Fisher, London, Ont.) I would like to point out that, according to the literature, most of the gastroduodenal ulcers seen in infants and young children occur in the duodenum. They are encountered infrequently in the stomach. I recently reported 4 cases of duodenal ulcers in young infants, all under the age of 8 months. The intriguing point to me was that most of these ulcers looked histologically very much like the chronic duodenal ulcers one sees in adults. They showed fibrosis in the floor of the ulcer, often endarteritis, and a defect in the muscular coat in the floor of the ulcer. As a matter of fact, in each of 2 cases in young infants, 3 months and 6 weeks of age respectively, an ulcer had perforated. In the first of the 4 cases I reported, 7 ulcers were present in the duodenum, 4 being distal to the ampulla of Vater, which is very unusual indeed, I think. I might add that in 2 of the 4 cases, in which the brain was examined, no cerebral lesions were found.

(Dr. Orville T. Bailey, Indianapolis, Ind.) In going over a series of approximately 60 cases of gastromalacia to see how constantly they were associated with intracranial lesions, I found severe lesions in the brain in all instances in which examination of the brain was permitted. In 2 patients permission to examine the brain was not obtained. I did not have as clear-cut clinical evidence of a cerebral lesion in those 2 cases as Dr. Schlumberger had in his patient in whom the head could not be examined. However, it is surprising to find how frequently cerebral lesions in children can be found at autopsy in the absence of clinical symptoms referable to them. One may assume that the association of cerebral lesions and gastromalacia is almost, if not wholly, complete. If this association is constant, there must be a definite physiologic connection, and the lesion in the stomach can hardly be put down as a true post-mortem change, even though it is not possible to demonstrate cellular infiltration. In 2 of my cases there was perforation of the esophagus with the result that a large amount of gastric contents escaped into the pleural cavity.

(Dr. Schlumberger) There seems to be little for me to add to these contributions by the discussants. The only question raised was the frequency of perforation of

the esophagus. In this series of cases there was only one instance in which this occurred.

THE RECOVERY PHASE OF ACUTE ANURIAS. Rafael Dominguez and (by invitation) Elizabeth Pomerene, Cleveland, Ohio.

Abstract. Functional recovery was studied in 9 cases of different anurias: shock nephrosis, dehydration nephrosis, hemolytic transfusion reaction, sulfonamide nephrosis, bichloride poisoning, and acute glomerulonephritis. In the beginning of recovery, when diuresis begins and the concentration of nitrogen metabolites of blood begins to fall, the urea nitrogen concentration in the urine is about three times the urea nitrogen concentration in the plasma. As improvement continues the concentration index of urea rises but is independent of diuresis. This anomalous situation continues until the concentration index rises to a value of 15 to 20. During this phase the urea clearance not only does not represent adequately the state of renal excretory function but may be wholly misleading. Subsequent improvement is characterized by the response of the concentration index to diuresis in the same direction as in the normal kidney, and from this point on the urea clearance reflects the renal functional improvement. With due allowance for this behavior of the urea clearance, the recovery of renal function as recorded by this test can be represented by one or two consecutive "S" curves. The approach of the upper part of the curve to its final stationary value is very slow. For this reason, it is impossible to determine the time at which the renal function returns to normal. It is possible, however, to determine rather sharply the time at which the clearance becomes 50 per cent of its normal value. If the beginning of recovery is placed at the peak of nitrogen retention, the 50 per cent recovery interval may be defined as the interval from the time of the nitrogen retention peak to the time of 50 per cent urea clearance.

In the cases of this study, the 50 per cent recovery interval varied between 14 and 21 days in shock nephrosis, 42 to 58 days in bichloride nephrosis, and 52 to 97 days in acute glomerulonephritis. In a case in which it was not possible clinically to decide between sulfonamide nephrosis and glomerulonephritis, the recovery interval was 32 days. In view of the results in the other cases, the diagnosis of sulfonamide nephrosis in this case is more likely than that of glomerulonephritis. A graph was presented to show the diuresis needed to maintain the blood urea nitrogen at a given level for different values of the concentration index.

THE TRIGGER MECHANISM OF ACUTE INFLAMMATION. Virgil H. Moon and (by invitation) George A. Tershakovec, Miami, Fla.

Abstract. There is not complete agreement concerning the factors which initiate the inflammatory reaction. The theory that "leukotaxine"—a substance obtained from purulent exudates—is the chief factor, merits further examination. Experiments were devised for studying the changes resulting after simple uninfected injuries. The agents used were local burns, local freezing of skin, intradermal injections of watery extracts of normal tissues, of turpentine, and of albumin into sensitized animals. The observation that these agents will induce inflammation is not new. Its significance lies in tissue injury as the trigger mechanism for inflammation.

In rabbits, 36 lesions were made by intradermal injections of muscle extract, 20 by injections of kidney extract, 10 by local burns, 10 by freezing, and 14 by injecting 0.02 cc. of 10 per cent solution of turpentine in sesame oil into the skin. In guinea-pigs, sensitized to albumin, 48 lesions were made by injecting albumin (0.4 mg. in solution) into the skin, 12 by local burns, 22 by local freezing (application of solidified CO_2), and 4 by injections of turpentine. In monkeys, 6 lesions were made by burning and 6 by injection of turpentine. Lesions so produced were examined histologically after intervals ranging from ½ hour to 24 hours. Acute hyperemia, edema,

and beginning migration of leukocytes developed within 30 minutes; these features were progressively more marked after longer intervals. In the beginning, the infiltrating cells were mostly neutrophils. Monocytes and lymphocytes became increasingly numerous within 24 hours. Capillary hemorrhages and diapedesis of red cells were present regularly. Fibrin was visible, even in the early stages of exudation. The number and variety of lesions studied seemed adequate, and the results were uniformly consistent.

The observation that cortisone inhibits the growth of granulation tissue raises the question whether it affects also the inflammatory reaction. Accordingly, a few animals were treated with cortisone prior to making cutaneous injuries. In these animals, the resulting hyperemia, edema, and leukocytic infiltration were not different in speed nor in degree from those seen in the untreated animals. Further experiments of this kind are being made.

Extracts of tissues have been shown to have chemotactic properties. These may have been a factor in the resulting leukocytic infiltration. But neither crystalline albumin, nor turpentine, nor sesame oil is known to be chemotactic. In the case of burns and freezing, no foreign substance was introduced, hence these reactions must have resulted from factors caused by the injury. Apparently, in these instances, the active agent was a substance derived from the damaged tissues. It has been shown that leukotaxine, in common with other biologic substances, will produce hyperemia, capillary permeability, edema, and infiltration with leukocytes. But it has not been shown that leukotaxine is present in normal tissues and that it is released from them when injured. Other workers have concluded that normal tissues, when damaged, liberate some substance which causes hyperemia, capillary permeability, edema, and leukocytic infiltration. Ebbecke, Lewis, and others believed that the cytoplasmic substance of injured cells was the substance concerned. Our results support that conclusion; apparently this is the "trigger mechanism" of the inflammatory reaction.

Discussion

(Dr. Valy Menkin, Philadelphia, Pa.) I think that Dr. Moon's slides are really beautiful slides of experimental inflammation and that is all, but I would appreciate knowing if he would extend his observations to say whether or not he has extracted leukotaxine from the exudates which he has obtained. This is of paramount importance in evaluating the leukotaxine theory. Without such evidence, the view becomes merely speculatory. In regard to the suppression of the inflammatory reaction by adrenal cortical extract and cortisone, we demonstrated this in 1940 and in 1942, but we have found recently that this does not always hold. For instance, if the exudate is at an alkaline level, then cortisone or adrenal cortical extract will suppress the reaction, but if the exudate is at an acid level there is no such suppression, but we found, however, that it can be suppressed by ACTH. In other words, ACTH will suppress another factor, termed exudin, concerned also in the mechanism of increased capillary permeability, and thus will suppress the reaction produced by acid exudate. I do advise Dr. Moon to use ACTH to see whether he can get any suppression of the inflammatory reaction with the irritants which he has used.

(Dr. G. A. Nedzel, Bethesda, Md.) I wish to add, in regard to the cortisone suppression of the hypersensitivity reaction of the skin to crystalline egg albumin, that in one experiment with rabbits 10 mg. of cortisone per day did inhibit the reaction, while ACTH was much less effective. Therefore I would like to ask Dr. Moon whether cortisone suppression was attempted in rabbits as well as in guinea-pigs and also what dose was used.

(Dr. William L. Robinson, Toronto, Ont.) In this work we should remember Abramson's work on physical chemistry in which he stresses electric fields where voltages are built up of 200 to 2,000 volts. Inflammatory cells are negatively charged

in electric fields, and the same thing with the red blood cells; they move to the positive pole. We hear very little about that in experimental work these days.

(Dr. Moon) I appreciate very much the discussion of this paper. I do not question Dr. Menkin's observations pertaining to the reaction of tissues, whether alkaline or acid. The chief point of difference in Dr. Menkin's interpretations and my own is that, before the injuries described, there were no leukocytes in the injured area. Since leukotaxine must be derived from leukocytes, it is difficult to understand how leukotaxine could be the agent causing the local inflammatory reactions. We believe that some substance derived from the tissues causes these reactions. That this may be related to leukotaxine is not doubted, but such a demonstration has not been forthcoming. The derivation of leukotaxine from the cytoplasm of injured cells would be highly interesting.

Concerning electric fields, we have made no experiments in that line. Our experiments on cortisone have been so few it would be unfair to prejudice your minds concerning it. Guinea-pigs were used and only 6 mg. of cortisone were given on the preceding day, in 2 doses, and 3 mg. on the day of the experiment, shortly preceding the injuries of the skin; perhaps those are inadequate amounts of cortisone. We are now experimenting with larger doses of cortisone under similar conditions.

ISOLATED MYOCARDITIS, MYOCARDIAL FIBROSIS, AND INTRACTABLE MYOCARDIAL FAILURE. Gustave J. Dammin and (by invitation) Robert J. Glaser and James C. Roberts, St. Louis, Mo.

Abstract. A clinical syndrome characterized by cardiac enlargement, low pulse pressure, and a prolonged course of intractable congestive failure occurring in the absence of valvular disease, hypertension, pericarditis, and coronary arteriosclerosis has been described by Smith and Furth, Davies, Levy, Dock, and others. The principal post-mortem findings have consisted of cardiac enlargement with subendocardial and myocardial fibrosis. Mural thrombi of the cardiac chambers with embolic lesions, usually involving the cerebral, mesenteric, splenic, renal, and pulmonary vessels, have been common. No cardiac or renal lesions usually associated with cardiac enlargement have been observed in these cases. Evidence that such a syndrome is related to chronic alcoholism and/or thiamin deficiency has not been convincing. A relationship to acute isolated myocarditis has been suspected.

During the last 4 years, 14 instances of this clinical syndrome have been observed; 9 of the cases have been studied post mortem. Eleven of the 14 were male patients. Most of the patients were in the 40 to 60 year age group. The average duration of the clinical course was 10 months. In 2 cases of relatively short duration cardiac enlargement was moderate; and extensive acute isolated myocarditis, myocardial fibrosis, and mural thrombi were observed at autopsy. Cases of longer duration showed myocardial fibrosis and mural thrombi only, with marked cardiac enlargement (700 to 800 gm.). The evidence suggests the fundamental lesion to be an isolated myocarditis of a recurrent or relapsing variety.

Those cases observed recently have been correctly diagnosed clinically because experience with earlier cases has produced an awareness of this clinical syndrome. Analysis of autopsy records suggests a recent increase in incidence of the syndrome. In the past, most cases have been diagnosed clinically as arteriosclerosis of the coronary arteries with myocardial infarction. Analysis of cases studied thus far has produced no clue as to the causative agents or processes involved. Earlier clinical recognition and investigation may yield such information.

Discussion

(Dr. Robert Fienberg, Framingham, Mass.) I would like to speak about the first group that Dr. Dammin has described, the patients with myocardial fibrosis. It is

very interesting that in cases of this type all the lesions in the heart where the muscle cells have disappeared have generally been called fibrosis. In a recent case of idiopathic myocardial fibrosis, much to my amazement, when I did special stains, both for collagen and for reticulum, many of these lesions were found to consist of little altered stroma with no collagen deposition. Thus, many of these lesions may be quite early or subacute. This point is important in understanding the clinical side because it is otherwise rather difficult to understand why some of these cases of myocardial fibrosis should die when they do with old fibrous lesions. Furthermore, these findings perhaps support the contention that myocardial lesions are a local necrotizing phenomenon due to a generalized metabolic disorder rather than sequelae of myocarditis.

(Dr. Jacob Werne, Jamaica, N.Y.) I have seen 2 cases of myocarditis in which microscopic studies of striated muscle showed trichinae in abundance. Was this possibility considered in the case presented?

(Dr. Virgil H. Moon, Miami, Fla.) Would it be out of place to suggest that the trigger mechanism which sets off the myocarditis in these cases has been anemic damage or necrosis of the muscle fibers, that in response to this injury leukocytes have been attracted to the area, and that the inflammatory reaction has thus been initiated?

(Dr. Dammin) The finding of fibroblasts and proliferating capillaries in the second group would make one suspect that it was a repair process in the form of fibrosis. It is certainly true that in the first group some of the lesions do suggest more a condensation of stroma rather than proliferation of reparative elements, but fibrosis appears to be the dominant lesion.

The comment by Dr. Werne reminds me of a similar question asked by Dr. Weller when such cases were presented in 1938. There was nothing in the clinical record of these cases to suggest that there was active trichinosis.

No anemia or evidence of vascular occlusion was observed during clinical observation of these cases, nor in the pathologic findings. We would relate the changes produced, perhaps to a direct action of a drug or an infectious agent or operating through the mechanism of hypersensitiveness. This is not necessarily a myocarditis produced by an infectious agent.

RHEUMATIC FEVER PNEUMONITIS (TWENTY-SIX NECROPSY CASES). Joseph F. Kuzma and (by invitation) M. J. Lustok, Milwaukee, Wis.

Abstract. The lesions seen in rheumatic fever pneumonitis are variable in distribution and in degree of development; however, the fundamental development is the same in all cases. It begins with an alteration of the morphologic and tinctorial character of the collagenous membranes of the alveolar septa, of the blood vessels, and of the larger interstitial supporting tissues. The histologic detail becomes obscured by a highly eosinophilic change. In the alveolar wall this is followed by exudation of fluid as well as by the extravasation of cells. At the same time there is reversion of the lining cells of the alveoli to a cuboidal character. This is frequently localized to the specific point of eosinophilic homogenization of the collagen membrane. Very shortly, these lining cells become large, deeply stained, and group into multinucleated giant cells and syncytial masses which show phagocytic activity. The fluid which exudes is rich in protein and frequently is closely applied to the alveolar wall forming a thick eosinophilic lining referred to as hyaline membrane. The cellular exudation includes red cells as well as polymorphonuclears, mononuclears, and lymphocytes. Such changes appear to be most prominent in the ducts of the atria. The eosinophilic protein material becomes attached to the underlying altered septa and becomes infiltrated by fibroblasts. Such structures have polar orientation of the fibroblasts and attach the material to the septal wall. These are known as Masson bodies. Changes also take place in the interstitial tissue and pleural spaces which are characterized by severe edema and lymphocytic and mononuclear cell infiltrations. Some eosinophilic smudginess of the collagen fibers in these zones is seen also. Blood

vessels are commonly involved by eosinophilic tinctorial change and occasionally by cellular infiltrations (principally polymorphonuclear cells) that produce destruction and the formation of abundant nuclear débris. This may involve parts of the vessel circumference, or the involvement may be diffuse and produce a pattern very similar to that of polyarteritis. Evidences of healing vascular reaction characterized by progressive intimal fibrosis and occlusive endarteritis are common. In one of our cases there was also evidence of similar eosinophilic necrosis of the basement membrane of the bronchial epithelium. These changes are not specific of rheumatic fever; they are observed in other conditions also, notably polyarteritis, subacute bacterial endocarditis, and lupus erythematosus. However, they are best developed in rheumatic fever and, therefore, are highly significant of this disease.

Discussion

(Dr. Norbert Enzer, Milwaukee, Wis.) In former years the reaction of the pleura in rheumatic pneumonitis was considered a significant feature of this condition. I would like to ask Dr. Kuzma if he has made any observation on the pleura in his cases.

(Dr. Charles E. Dunlap, New Orleans, La.) We have had 2 autopsies recently, on patients diagnosed clinically as having acute rheumatic fever. Pneumonitis was found microscopically quite similar to that described, but no typical lesions were found in the myocardium or endocardium. I would like to ask if all of the cases reported showed the characteristic reaction of rheumatic fever in the heart.

(Dr. Henry W. Ferris, Ithaca, N.Y.) Were any cases studied clinically which were not autopsied; if so, what was the distribution of lesions, and how long did they last?

(Dr. Kuzma) We looked at the interstitial tissue and pleura, and some of the changes as previously noted in published papers and textbooks were confirmed. However, in none of these areas did we find anything that was specific for the Aschoff nodule, although histiocytic cells and various changes were found in all portions. The important thing is that these cells did not aggregate into a unit as they do in the myocardium.

Dr. Dunlap, in reply to the question of myocardial changes, we have some instances in which the valves showed minor rheumatic involvement which we considered active, and myocardial sections did not show any particular change. However, on further sectioning and repeated sectioning, we were able to find, in each of our instances, the presence of active rheumatic myocarditis accompanied by distinguishable Aschoff nodules. In most of our cases it has not been difficult, and it is true that an active rheumatic myocarditis has been coexistent with the changes in the lungs.

Dr. Ferris, in reply to the question of clinical data: we do not have particular information on that. All of the material we are talking about is from autopsy cases. The roentgenographic changes were variable, and one of the particular features is that the findings in the lungs which were interpreted as infiltration were evanescent, and may be evanescent in a matter of hours. They extend through the entire course of the disease in variable periods and usually are seen many times during the illness.

HISTOPATHOLOGIC STUDY OF ACUTE NON-FATAL HEPATITIS. Theodore C. Keller (by invitation), Burton Giges (by invitation), and Hans F. Smetana, Washington, D.C.

Abstract. In 20 cases of acute non-fatal hepatitis a needle biopsy of the liver was performed from 3 to 12 days after the onset of symptoms. In addition, a second biopsy was performed after an interval of from 10 to 20 days in 5 of the cases. The lobular pattern of the liver was unaltered. The most prominent feature observed in the initial biopsies was diffuse scattering of mononuclear cells throughout the lobules, usually within the sinusoids, but also in the form of small aggregates which were

interpreted as foci of necrosis. The portal canals usually contained an increased number of these cells, often accompanied by a few eosinophilic leukocytes. The reticulum was intact, and neither scarring of the portal canals nor increase in the number of small bile ducts was seen. The Kupffer cells were prominent and often contained a very finely granular, yellow-brown pigment, which was interpreted as lipochrome derived from necrotic parenchymal cells. In addition, some of them contained small droplets of fat. Parenchymal cells with large, hyperchromatic nuclei or double nuclei were frequently noted. Mitotic figures were demonstrated in 10 cases. In all instances solitary cells composed of an acidophilic cytoplasm, with or without a small pyknotic nucleus, appeared to be lying free within the sinusoids, without evidence of zonal predominance. Such cells have been described previously in cases of acute hepatitis, and interpreted as necrobiotic liver cells.

A comparison of the first and second biopsies revealed consistent differences: in the second biopsies relatively few mononuclear cells were seen within the portal canals or the lobules. The number of acidophilic cells was greatly reduced. Pigmented Kupffer cells and regenerating parenchymal cells were quite prominent. Foci of mononuclear cells were more sharply defined and constituted the most consistent histologic change observed in the second specimens. A small amount of bile pigment within the bile capillaries was clearly visualized in the initial biopsies in only one case; however, in 2 instances the second biopsy revealed small masses of bile pigment diffusely distributed throughout the lobules. Significant histologic alterations were observed even in clinically mild cases, indicating that the histologic appearance was not an accurate index of the clinical severity of acute non-fatal hepatitis.

PATHOGENESIS OF DISSECTING ANEURYSM OF THE AORTA. Ira Gore, Detroit, Mich. (Study made at Armed Forces Institute of Pathology, Washington, D.C.)

Abstract. Medial degeneration as described by Gsell and Erdheim formed the pathologic basis of 85 dissecting aortic aneurysms studied at the Armed Forces Institute of Pathology. A parallel seemed to exist between the localization of lesions in the proximal aorta and the metabolic gradient required for maintenance of structural integrity of the aortic media. Any interference with the metabolic cycle theoretically would eventuate in retrogressive structural changes. In 18 of 32 individuals younger than 40, the predominant association of cardiovascular congenital anomalies with medial degeneration and dissecting aneurysm suggested that an inborn defect might form the basis of one type of metabolic difficulty. Since hypertension imposes an additional burden upon the vascular tree, it would operate as a contributory but not essential factor. Elevated arterial pressure had not been present in 75 per cent of those younger than 40 years.

Medial degeneration was accompanied by reactive changes; namely, accumulation of poorly cellular myxomatous material, new-formed vasa, and a mild lymphocytic infiltrate about the vessels. Vascularization was remarkably consistent, and provided the source of the intramural hemorrhage which, it is believed, constituted the first step in the formation of a dissecting aneurysm. There is no other way of explaining the occurrence of dissecting aneurysm without an intimal break. Syphilis had been ruled out clinically, serologically, and pathologically. In contrast to syphilis, the bland inflammatory process in idiopathic medial degeneration results in thin-walled, widely patent vessels supported by the structurally inadequate tissue of the medial lesion. Such vessels are visibly vulnerable to rhexis, a possibility which the presence of hypertension enhances. Hemorrhagic cleavage of the diseased media proceeds under arterial pressure which, after intimal rupture, is admitted directly from the aortic blood stream; exceptionally the entire process remains intramural. Interruption of nutrient channels leads to ischemic necrosis and further medial weakening. There was no evidence that ischemia played a more primary rôle in the genesis of the initial medial lesion. Localization of the initial hemorrhage in dissecting aneurysm

seems well explained not only by the greater frequency of lesions in the proximal aorta but also by the greater mechanical stress to which that segment is subject.

CHRONIC ENCEPHALITIS WITH INTRANUCLEAR AND INTRACYTOPLASMIC INCLUSIONS. David E. Smith (by invitation), S. A. Trufant (by invitation), Vol K. Phillips (by invitation), and Margaret G. Smith, St. Louis, Mo.

Abstract. A case of chronic encephalitis is described in which there were extensive areas of cortical and subcortical destruction with intranuclear and intracytoplasmic inclusions in neurons and oligodendroglia. The pathologic lesions were similar to those described in 2 cases by J. R. Dawson and subsequently in 8 cases by other authors. The patient was a white boy, 16 years of age, whose illness began 7 months before death with attacks of dizziness and ataxia followed by irrationality and frequent convulsions. The cerebrospinal fluid contained no abnormal constituents. In the brain at autopsy the lesions consisted of areas of destruction of neurons and many neurons that contained intranuclear inclusions. A few neurons with intranuclear inclusions also had smaller intracytoplasmic inclusions, and some intranuclear inclusions were present in oligodendroglia. In the affected cortex and subjacent white matter there were concentrations of fat-laden macrophages and hypertrophied astrocytes with an accompanying perivascular infiltrate of round cells. Except in the cortical and adjacent subcortical foci involved by the active destruction, there was no destruction of myelin or apparent degeneration of tracts. The lesions were present in the cortex of all lobes of the cerebrum but were most extensive in the left precentral region. Brain substance collected at autopsy was preserved by freezing for 3 weeks and then inoculated intracerebrally into young adult Swiss white mice and onto the chorioallantoic membrane of embryonated eggs. The material from the intracerebral inoculations was transferred through 3 groups of mice and that from the eggs through 2 groups of mice without demonstration of a causal agent.

THE EFFECT OF HEMOLYTIC STREPTOCOCCUS TOXIN ON EXPERIMENTAL VIRAL CARDITIS. John M. Pearce, New York, N.Y.

Abstract. During the past several years it has been shown that the heart of the rabbit is susceptible to infection by filtrable viruses when these agents are introduced into the periphery of the animal body by intranasal, intratesticular, or cutaneous routes. In the heart the virus gives rise to an inflammatory reaction involving chiefly the muscle but affecting also the pericardium and endocardium. The incidence and the severity of the lesion are greatly increased if, preceding, or concurrently with, the inoculation of the virus, the rabbit is subjected to some procedure that decreases the volume of oxygen supplied to the heart muscle. That cardiac hypoxia was the determining factor in the localization of virus in the heart was first indicated by the effects of drugs that caused constriction of the coronary arteries, and then directly substantiated by placing the virus-infected animals in an atmosphere deficient in oxygen. Because of the similarity of the experimental viral lesions to those in hearts affected by rheumatic fever and the suspected rôle in the latter disease of the hemolytic streptococcus, the experiments reported here were done to determine whether the toxin from this organism also induced cardiac localization of virus.

Two groups of 20 rabbits were given a single intravenous injection of varying amounts of the oxygen labile hemolysin of *Streptococcus pyogenes*. Immediately thereafter one of the groups was inoculated intratesticularly with virus III. This infecting agent was chosen because of the typical intranuclear inclusion bodies that characterize and identify the lesions caused by it. All animals were sacrificed 4 to 8 days after infection and their hearts were examined for inflammatory lesions with and without viral inclusion bodies. Of the animals that received virus and toxin, 14 (70 per cent) had severe cardiac lesions and in 9 (45 per cent) there were easily demonstrated inclusions. The 5 without inclusions were killed on the eighth day,

at which time these structures have usually disappeared. Myocarditis predominated but the mural endocardium was frequently thickened and infiltrated with leukocytes and in one case there was a fibrinoid vegetation on the tricuspid valve. In the hearts of 8 of the rabbits (40 per cent) that received toxin only, there were mild alterations consisting of sparse, diffuse or focal infiltrations of lymphocytes in the myocardium and occasional, single, scattered, acidophilic, necrotic muscle fibers. Only the necrotic muscle can be attributed to the effect of the toxin with any certainty. Further controls were furnished by 74 rabbits inoculated with virus but otherwise untreated, in only 12 per cent of which a mild viral myocarditis occurred. The results from these animals were collected during previous studies.

It can be concluded that the administration of the hemolytic toxin of *Streptococcus pyogenes* increases the incidence and severity of lesions in experimental viral carditis. The mechanism by which this occurs may be a vasoconstrictor effect of the toxin on the coronary arteries producing cardiac anoxia, or it may be a direct necrotizing action of the toxin on single muscle fibers thereby producing a focus of decreased resistance and allowing the virus to become established in the myocardium. That the degree of intravascular hemolysis does not parallel the severity of cardiac lesions makes it improbable that hypoxia brought about in this way is responsible.

Discussion

(Dr. Edward C. H. Schmidt, Philadelphia, Pa.) I would like to ask Dr. Pearce if the lesions appeared in other organs, particularly in the central nervous system, and what was the rate of spontaneous myocarditis in the race of rabbits he used.

(Dr. Pearce) To the first of these questions, whether there were lesions in other organs, lesions did occur at the site of inoculation; if the rabbit was inoculated intratesticularly, there was a marked orchitis full of inclusion bodies. When the virus was injected into the skin there was a minimal lesion. When it was injected subcutaneously the virus was absorbed from the skin without producing much of a lesion there, and when it was done intranasally, there was occasionally, in a small number of cases, a slight pneumonitis, but the rule is for the virus to be absorbed through the lung and not to produce any lesions. This applies to virus II. Virus III is a very mild one which never kills; it runs a clinical course of 10 to 12 days, and the rabbit runs a fever on the 4th to the 6th day.

As to the central nervous system, one would expect that the central nervous system would be the most susceptible of all parts of the body to the hypoxia, but we have never found any lesions in the central nervous system. We have found lesions in hilar lymph nodes and in other regional nodes. We have also, curiously, not infrequently found small necrotizing lesions in the adrenals in which there were many easily recognizable inclusion bodies.

The incidence of spontaneous cardiac infection—by that you mean when virus is injected?

(Dr. Schmidt) I mean in the routine animal, without any treatment at all.

(Dr. Pearce) In my stock it is very rare, and we use very young animals.

THE INCIDENCE, DISTRIBUTION, AND SIGNIFICANCE OF MEGAKARYOCYTES IN NORMAL AND DISEASED HUMAN TISSUES. Edward B. Smith, St. Louis, Mo., and (by invitation) James Butcher, Philadelphia, Pa.

Abstract. There is sometimes confusion regarding the identity and significance of large cells with lobate nuclei and of large, dark nuclei with scant cytoplasm observed in the capillaries, especially in patients showing hemorrhage, thrombosis, blood dyscrasia, recent pregnancy, or malignant tumor. It has been shown previously that megakaryocytes occur in the circulating blood and can be demonstrated microscopically in tissue sections. The present study yields basic quantitative data from analysis of sections from 79 control cases of "sudden" death (accidental, cardiac, pontine

hemorrhage) and from 111 hospital cases who died "slowly." The number of megakaryocytes was determined in microscopic sections (approximately 0.5 cmm. of tissue), one each of heart, lung, liver, spleen, and kidney from each case. The number of megakaryocytes per cmm. of bone marrow was determined in 36 control cases and in 108 hospital cases.

In the 79 cases of sudden death, megakaryocytes were found in 90 per cent of the spleens (averaging 3.5 such cells per section); 88 per cent of the lungs (averaging 5 cells); 35 per cent of the kidneys; 24 per cent of the livers, and 16 per cent of the hearts. The "average normal" marrow section presented 2,970 megakaryocytes per cmm. (approximately 134 per 100 "high-power fields"). In the 111 cases of hospital death, megakaryocytes were found in 100 per cent of the lungs (averaging 14 such cells per section); 97 per cent of the spleens (averaging 7 megakaryocytes per section); 71 per cent of the kidneys; 66 per cent of the livers; and 45 per cent of the hearts. The bone marrow of 108 cases averaged 3,766 megakaryocytes per cmm. (approximately 170 per 100 "high-power fields"). These counts represent an increase over the normal for every organ. The cases of hospital death with significant infection showed the greatest increase in megakaryocytes, whereas those with cirrhosis tended to display a decreased incidence in tissues and bone marrow. Cases showing hemorrhage, thrombosis, malignant tumor, and chronic cardiovascular disease conformed consistently to the average for all of the hospital cases. The average megakaryocyte content of normal bone marrow decreased with advancing age, but no correlation with age was demonstrated in ill persons.

BONE MARROW EMBOLISM. J. H. Fisher, London, Ont.

Abstract. Bone marrow embolism occurs much more frequently than has been reported previously. In a survey of 96 unselected cases of fractures, 19 cases (19.8 per cent) of bone marrow embolism of the pulmonary arteries were found. The emboli were few in any particular case and might have been easily overlooked. Apparently they have been overlooked in the past. They obstructed so few pulmonary arteries that significant disturbances did not occur in the lungs. Therefore they are of no practical importance. Of the 19 cases, 13 were males and 6 females. Their ages varied from 7 to 86 years. There was no predilection for any particular part of the lungs. In the various cases, the right lower lobe, the right upper lobe, the left upper lobe, the left lower lobe, and the right middle lobe were involved in that order of frequency, but actually the distribution of emboli is such as to have no significance. The embolism cases survived from a few minutes (almost instantaneous death) to 6 days after the injuries were inflicted. In one case the skull alone was fractured, but in most cases multiple bones were fractured. The ribs, pelvis, vertebrae, tibia and fibula, skull, and sternum were the bones most frequently involved in that order. In most cases the persons were subjected to very forceful violence in automobile accidents.

Discussion

(Dr. Russell L. Holman, New Orleans, La.) I would like to ask what the incidence of fat embolism was—whether there is any correlation between fat embolism and bone marrow embolism.

(Dr. P. O'B. Montgomery, Boston, Mass.) That bone marrow embolism may be of academic interest in the case of normal bone marrow I agree; however, should one be dealing with a fatty marrow, such embolism may be fatal. I have in mind the case of an elderly anemic female with rheumatoid arthritis upon whom a sternal bone marrow aspiration was performed with a Turkel needle. Six hours later she was dead. At autopsy fat embolism of the lungs was demonstrated, in the sections of which were bone marrow units. The sections of the marrow showed considerable fat replacement.

(Dr. Henry Rappaport, Washington, D.C.) We have recently completed a study

of 27 cases of bone marrow embolism * from the files of the Armed Forces Institute of Pathology, and our observations are in essential agreement with those of Dr. Fisher. When Lubarsch, in 1898, reported the first instances of bone marrow embolism in patients who had died from eclampsia, he did not know that generalized convulsions may cause vertebral fractures. It was not until 1907 that Lehndorff first recognized spinal deformities in a patient who had recovered from tetanus. His findings were later confirmed by others who demonstrated a high incidence of vertebral fractures following tetanus and convulsive shock therapy with insulin or metrazol. For this reason, in addition to 200 cases of accidental death with multiple fractures, we examined lungs from cases of post-convulsive death and were able to demonstrate pulmonary bone marrow embolism in tetanus, eclampsia, convulsive shock therapy, accidental electrocution, and convulsions due to other causes. In these cases fractures were neither suspected nor demonstrated. It must be realized, however, that vertebral fractures may be easily overlooked at necropsy. I should like to add that bone marrow embolism may be of more than academic interest. By means of large sections of entire lobes of lung we have been able to demonstrate massive embolization in 2 cases in which both lungs were still available *in toto* after pulmonary bone marrow emboli had been discovered in routine sections. In one, bone marrow embolism was the only finding to account for the patient's death.

(Dr. Fisher) In reply to Dr. Holman's question, I think that in most of the cases fat embolism probably did occur with the bone marrow embolism. I did not do fat stains on the lungs in these cases, so I cannot answer that question accurately. I am quite sure fat embolism was associated with bone marrow embolism in many of the cases, perhaps in the majority. On the other hand, I am quite convinced that bone marrow embolism did occur in some of the cases unassociated with fat embolism and of course fat embolism occurred alone in others.

I am interested to hear what Dr. Montgomery has said about the possibility of bone marrow embolism being fatal.

I am very grateful to Dr. Rappaport for bringing to my attention the 27 cases of bone marrow embolism which he has found in the material at the Armed Forces Institute of Pathology. I examined sections of the lungs in 3 cases of eclampsia but failed to find bone marrow emboli. However, I cannot say whether vertebral fractures were present in those cases. I thank Dr. Rappaport also for the additional interesting information which he has given.

ANOMALIES OF THE MAJOR CEREBRAL ARTERIES ASSOCIATED WITH CONGENITAL MALFORMATIONS OF THE BRAIN, WITH PARTICULAR REFERENCE TO THE PATHOGENESIS OF ANENCEPHALY. F. Stephen Vogel (by invitation) and John L. McClenahan (by invitation), New York, N.Y.

Abstract. As a first step towards learning more about the etiology and pathogenesis of congenital cerebral malformations, detailed post-mortem examinations were made of 9 anencephalic monsters. In all cases the cerebral hemispheres were replaced by masses of vascular fibroglial tissue containing rudimentary choroid plexuses and a few poorly differentiated neural elements. Well formed optic nerves together with retinae and eyes were present in each case, the findings making it plain that the central nervous system had developed normally during the first 5 weeks of life. In 7 of the cases there were well developed medullae with complete spinal cords, spinal nerves, and root ganglia. It seemed noteworthy that the cerebral anomalies occurred regularly in the region normally supplied by the internal carotid arteries, while the well formed parts received their blood supply from the ophthalmic and vertebral arteries. Furthermore, the presence of glial and mesenchymal tissues and the paucity of the more specialized neural elements suggested that impaired vascularity may have been responsible for the malformations.

* *Am. J. Path.*, 1951, 27, 407–433.

To study further the relationship between vascular anomalies and congenital malformations of the brain, a new technic has been devised whereby a fusible metal is injected into the arch of the aorta of the warmed cadaver; the cerebral vessels are thus filled with the fused metal, which hardens when cooled. The metal serves as an excellent contrast medium for x-ray studies; furthermore, the soft tissue can be digested away leaving rigid metallic castings of the arterial system. This technic has been applied in a study of 4 cases of congenital cerebral malformations. In each instance marked abnormalities of the large arteries of the circle of Willis were evident.

To learn whether vascular anomalies induced at a suitable stage of development will be followed by cerebral malformations, the internal carotid arteries of developing 6-day-old chick embryos were occluded by electrocauterization. When the embryos were sacrificed 7 days later, a marked retardation of cerebral development, not unlike anencephaly in human beings, was evident on gross and microscopic examination. The findings as a whole indicate that anomalies of the major cerebral arteries of the brain may have importance in the pathogenesis of anencephaly.

Discussion

(Dr. Peter Gruenwald, Brooklyn, N.Y.) I think there is one feature of anencephaly which I am not sure has been reproduced in the chick embryo experiments, and that is that the neural groove remains open; I think this should also be considered. There is, following the occlusion of the blood vessels in the chick embryo, some degeneration of nervous tissue; in the human there is similar degeneration due to maceration by amnotic fluid. I wonder whether we will not have to consider further the possibility that the main process in the human is a failure to close, and whether the vascular changes are not secondary.

(Dr. Vogel) In the experiments with chick embryos we investigated the cause and effect relationship between the vascular anomalies and the congenital malformations of the brain observed in cases of anencephaly in human beings; the results of these studies merely indicate that the vascular lesions could be primary and the cerebral changes secondary. However, I believe it has been clearly demonstrated that a diminution in the cerebral blood supply in chick embryos regularly results in an arrest or retardation in the development of the brain. If the neural groove is not closed when the alterations in the blood vessels occur, it might be expected that closure of the neural crests would not follow.

SYMPOSIUM ON THE RELATION OF THE ADRENAL GLANDS TO SYSTEMIC DISEASE

SECRETION OF GLUCONEOGENIC AND LIPID HORMONES. Frank A. Hartman (by invitation), Columbus, Ohio.

Abstract. Because so much of our information regarding the secretory activity of the adrenal cortex is based on indirect evidence, we have begun a study of cortical activity by collecting blood from the adrenal gland and determining the hormones secreted. Blood was collected continuously from dogs under nembutal for periods as long as 6 hours. The plasma was extracted with ethylene dichloride and the hormones finally taken up in isotonic sodium chloride solution. The gluconeogenic hormone was determined by its ability to cause deposition of glycogen in the liver in adrenalectomized mice starved 24 hours, while the lipid hormone was determined by its ability to cause deposition of lipid in the liver of 24-hour starved, adrenalectomized mice. The amount of gluconeogenic hormone secreted per unit of time varied greatly from one animal to another, but there was always some secretion. It was lowered by temporary occlusion of the blood flow. Epinephrine even in large doses produced no effect on the secretion while ACTH caused a marked increase.

Comparison of adrenal and arterial blood showed that the adrenal gland is the seat of production of the lipid hormone. The amount of lipid hormone secreted varied greatly, from values too small to detect by our method of testing to a considerable amount. Temporary occlusion of the blood flow reduced the production of lipid hormone. Transfusion with isotonic sodium chloride solution also reduced it. Epinephrine injected did not influence the secretion. ACTH was without effect, although the same dose caused a marked increase of gluconeogenic hormone secretion in the same animal. We were unable to show that alimentation produced any effect. It is concluded that the lipid hormone is distinct from the gluconeogenic hormone. Our evidence indicates that the amounts of gluconeogenic and lipid hormones secreted per hour are many times the quantities found in the adrenal gland at any one time. In other words, the glands must be more or less continuously active to furnish the required hormone since there is little or no storage.

A Histopathologic Study of the Human Adrenal Gland in Patients Receiving ACTH and Cortisone Treatment. W. A. Bennett, Rochester, Minn.

Abstract. This study covers approximately 18 cases of various conditions treated with cortisone and ACTH. The adrenal glands are compared with a similar series of normal individuals dying of accidents and other disease conditions. Special stains were undertaken and evaluated.

Discussion

(Dr. Chester Solez, Buffalo, N.Y.) Were definite changes found in the pituitary as well as in the adrenal glands in these patients?

(Dr. G. H. Klinck, Washington, D.C.) I would like to know more concerning the usefulness of the Ashbel-Seligman reaction. Do you feel that further refinements of the test will lead to better correlation of the changes in the adrenal glands?

(Dr. Jesse L. Carr, San Francisco, Calif.) Will Dr. Bennett discuss the possibility that these persons who had diseases helped by ACTH and cortisone had an adrenal hypoplasia to start with?

(Dr. Norbert Enzer, Milwaukee, Wis.) Has Dr. Bennett studied adrenal glands from similar conditions before the era of cortisone and ACTH to see how the adrenal glands in these conditions compared with glands subjected to ACTH and cortisone therapy?

(Dr. Katharine Brownell, Columbus, Ohio) I should like to ask if Dr. Bennett has found any dose of cortisone beneficial in a given clinical condition which does not produce cortical atrophy?

(Dr. Peter Gruenwald, Brooklyn, N.Y.) I would like to know whether one finds after the use of ACTH evidence of over-stimulation leading to disintegration of cortical cells.

(Dr. Bennett) Due to the short time for the presentation there were many things I did not mention. As far as the pituitary body goes, we did find changes. There is a paper which is going to follow by Dr. Laqueur, and I am limiting my remarks to the adrenal cortex. We found changes with both cortisone and ACTH, the so-called Crooke's change.

In regard to the Ashbel-Seligman reaction, I think that is a crucial test, and we have just set up a freezing dehydration apparatus to carry it out. I am not convinced at the present stage that it is a specific thing, but I think there are possibilities in the test; it should be carried out, and I think it may be helpful. I think masses of lipid materials are a mixture, and if we can separate one small mass of lipid material from another it will be helpful for the morphologist. We have to go to the histochemical methods which were described here by Stowell; these are the crucial points.

In reply to the question whether I have compared the adrenal glands with those before the era of ACTH and cortisone, I have looked at 4,000 trying to get a base

line, and in none of these did we find any changes which simulated the atrophy we see with cortisone. This is not so in ACTH stimulation, for there are several things that may stimulate the adrenal gland and give a pattern which is very similar. I have had the men in the laboratory bring me adrenal glands as unknowns and I think I can pick out the ACTH glands from the ordinary ones by studying them carefully. It takes time and experience, but I think it can be done, and with some of these special tests we may find something which is going to help us in telling whether an adrenal is ACTH-treated or stimulated from some other source.

As far as these being hypoplasias from the start, I have not found any to this degree. I think there is a depletion in starvation and other debilitating diseases, but we found much sudanophilic material scattered throughout the thickness of the cortex.

As to the dosage, we had one individual who had 25 mg. over a long period of time, and that was helpful in relieving his asthma; an asthmatic attack was the cause of death, and there was marked atrophy in this case. We have no indication whether small doses of short duration have any effect on the adrenal gland, therefore I cannot answer the question, because those individuals do not die, and we have none of this material available at this time.

EXPERIMENTAL OBSERVATIONS WITH MASSIVE DOSES OF CORTISONE. William Antopol, New York, N.Y.

Abstract. The daily administration of massive doses of cortisone to mice produces a striking lymphopenia, loss in body weight, atrophy of thymus and spleen, diminution in size of the adrenal cortex, anterior pituitary gland, salivary glands, and hibernating fat bodies, as well as an increased susceptibility to low environmental temperature and infections. In 20 per cent of the mice treated with cortisone, focal granulomata were present in the liver, kidney, heart, and/or hibernating fat bodies. These granulomata were composed of nuclear débris and closely aggregated leukocytes; occasionally clumps of bacteria were present at the periphery without appreciable reaction. Fibroblasts were inconspicuous or absent. The lesions were sharply delineated from adjacent structures, but parenchymal cells adjacent to the abscesses showed degeneration and necrosis. *Corynebacterium pseudotuberculosis murium* was cultured from most of these mice. These changes were not considered to be due directly to cortisone, but secondary to the altered reaction pattern of the mice, together with the infection to which they were now susceptible. When 0.1 cc. of a 24 hour broth culture of *C. pseudotuberculosis murium* was inoculated subcutaneously, only 2 of 15 control mice developed macroscopic abscesses in the organs, and these were in the lungs. In contrast to this, following subcutaneous administration of the organisms to mice after 2 days of pre-treatment with cortisone (2.5 mg./20-25 gm. mouse), gross abscesses were found in 6 of the 11 mice; 5 had liver abscesses, 4 kidney abscesses, 3 heart abscesses, and 5 lung abscesses.

The literature reveals that cortisone influences the protein, carbohydrate, and fat metabolism, affects enzyme systems, modifies the reaction of the body to physical and chemical agents and to bacteria, and produces lymphatic atrophy, lymphopenia, eosinopenia, and a fall in circulating antibodies. The mechanism of these multiple effects is not clear. It is unlikely that all of the cortisone effects are attributable to a common denominator. However, some of the effects are probably manifest through the catabolic and possibly anti-anabolic effect of cortisone on proteins, particularly gamma globulin. This could explain the inhibiting effect of cortisone on the growth of immature mice and rats, the weight loss of cortisone-treated animals, the inhibition of certain enzyme systems, the fall of antibodies in immunized animals receiving cortisone, and the poor response in the development of antibodies when cortisone is given during immunization. In addition to this effect of cortisone on protein, it also produces atrophy of the adrenal cortex and anterior pituitary lobe. The suppression

of adrenal activity may become evident clinically not only after cortisone is discontinued, but also during its administration, since the atrophic cell may be concerned not only with cortisone production but also with the production of other necessary steroids.

Because cortisone, particularly through its protein catabolic effects, may affect so many systems, it is beneficial in a great number of maladies. On the other hand, substances which modify only one or two factors will be helpful in only a small percentage of these diseases or in a limited number of cases of the same disease.

Discussion

(Dr. W. A. Bennett, Rochester, Minn.) We had an interesting experience in one patient with leukemia treated with cortisone, a 2½-year-old girl who developed lactation on the hormone, also mature endometrium, mature vaginal epithelium, and development of ova in each ovary. This fits in with the development of increase of size of the mammary gland.

(Dr. Antopol) It is very interesting in the light of Dr. Bennett's findings that in mice there is retardation with a tendency to luteinization instead of rapid maturation. In male immature mice the testes did not mature fully. An abstract of the breast changes following massive doses of cortisone which was added to the presentation will appear elsewhere.

THE EFFECT OF ACTH AND CORTISONE ON THE HUMAN ADRENAL GLAND. L. Sokoloff and (by invitation) J. T. Sharp and E. H. Kaufman, New York, N.Y.

Abstract. In a systemic investigation of the human adrenal glands in rheumatic diseases, the weights, cholesterol content, and quantitative measurements of the zonal architecture of the cortices have been determined. To date 117 pairs of adrenal glands have been studied; these include 28 in which variable doses of ACTH or cortisone have been administered. The influences of the larger doses of these drugs on the adrenal glands as compared with control glands are summarized. The observations indicate that large doses of ACTH induce hypertrophy of the adrenal cortex with diminution of the cholesterol content; these changes disappear when this medication is withdrawn. Cortisone may cause atrophy of the cortex that also is reversible upon cessation of therapy.

Discussion

(Dr. Tracy B. Mallory, Boston, Mass.) I should like to ask one question. In the table of weights of glands one group was listed as derived from traumatic deaths. Were those cases in which trauma produced relatively sudden and immediate death, or were they cases in which trauma was followed by a shock-like state, perhaps an agonal state lasting several hours before respiration finally ceased?

(Dr. Sokoloff) The cases of traumatic death were obtained from the Medical Examiners Department in the City of New York. In all but one or two, death was immediate; in these the patient died within 20 to 30 minutes.

ADRENAL CORTICAL SUBSTANCES, ACTH AND SEX HORMONES AND THEIR INFLUENCE ON THE THYMUS GLAND. Jesse L. Carr, San Francisco, Calif.

Abstract. ACTH and gonadotropic hormones specifically involute the thymus and their absence or inadequacy results in thymic hyperplasia. In the so-called "thymic status" there is an anatomical parallelism and physiologic similarity which we believe to be important.

Discussion

(Dr. Carl V. Weller, Ann Arbor, Mich.) I wish to call attention to the fact that in addition to the Pacific coast there is another region where there have been unpopular proponents of the status thymicolymphaticus, and that is in southern Michigan.

For 30 years I have taught that there is a reciprocal relationship between the adrenal gland and thymus and, having emphasized that it is the small size of the adrenal gland and not the large size of the thymus which is important in the thymicolymphatic constitution, the developments of the last few years permit me to die happy.

(Dr. Jacob Werne, Jamaica, N.Y.) I am happy that Dr. Carr's experience in presenting material to this Association has led him this time to abandon status thymicolymphaticus as a cause of death. However, in substituting asphyxia of undetermined etiology, he is probably standing on ground that is not any more secure. It is important that the pathologic anatomist be critical of the nomenclature used in assigning a cause of death. We know that apart from the asphyxiating instrument or cause, there is no pathognomonic evidence for asphyxial death. For example, if Dr. Carr will again review the section from which he projected the microphotograph showing occlusion of the bronchus with exudate he will, for example, find there an adequate cause of death in the form of occlusive suppurative bronchiolitis. Those cases for which a reasonable cause of death is not found should be certified as cause of death undetermined.

(Dr. Henry W. Edmonds, Washington, D.C.) The believers in status thymicolymphaticus not only die happy, but they die hard.

(Dr. Carr) There is little to say except that there is no defense at all for status thymicolymphaticus except the evidence which is shown. I might attempt to reverse the remarks of those who oppose the very frankly demonstrated evidence of a very interesting chain of events between pituitary gland, adrenal gland, and thymus and suggest that those who are unable to make this association are already dead.

PITUITARY CHANGES IN DISEASES OF THE ADRENAL CORTEX. G. L. Laqueur, Bethesda, Md.

Abstract. The reciprocal relationship between the adrenal cortex and the hypophysis is well established. Experimental observations suggest that this relationship consists of a delicate balance between pituitary ACTH and the level of circulating adrenal cortical hormone (Sayers). The paper presents observations on human hypophyses in cases of reduced and increased levels of circulating cortical hormones.

The effect of decreased adrenocortical function upon the hypophysis was studied in cases of Addison's disease resulting from cytotoxic destruction of the adrenal cortex. In this condition, groups of large non-granulated cells are frequently encountered in the anterior lobe, and they can be identified as beta cells by their content of material positive with the PAS reaction. Loss of granulation, vesicular and smooth nuclei, and an abundant cytoplasm containing small amounts of Schiff positive material were taken as evidence for increased activity of the beta cells. Examination of hypophyses obtained from routine autopsy material indicates that similar anterior lobe changes suggesting activity of beta cells may be found in myxedema and in a small number of individuals in whom depletion of cortical lipid is severe. The pituitary changes resulting from elevated levels of circulating adrenocortical hormone are demonstrated on glands of individuals with Cushing's disease in which the granules of the beta cells frequently are replaced by a hyaline material (Crooke's hyaline change). This material has the same staining properties as the beta cell granules and is "Schiff positive." Similar cytoplasmic changes can be produced experimentally by massive doses of adrenal cortical hormone, lending support to the theory that the hyalinization of the cytoplasm of the beta cells in Cushing's disease is secondary to increased levels of circulating adrenocortical hormone.

Discussion

(Dr. Chester Solez, Buffalo, N.Y.) I would like to ask if Crooke's changes represent hyperactivity or hypoactivity in the pituitary gland.

(Dr. J. F. A. McManus, Charlottesville, Va.) In this paper at one point Dr.

Laqueur used "basophil" as synonymous with beta cells. I think basophilia depends on the stain; the hematoxylin and eosin stains, for example, do not always give true separation into acidophils corresponding to alpha cells and basophils corresponding to beta cells. We have had one case of Addison's disease following diabetes, complicated by myxedema, in which there were large groups of cells in the anterior pituitary gland which, after Zenker's fixation, had relatively little PAS-positive material in the cells themselves, but the cells seemed to be grouped into a pseudo-acinar arrangement in which there was in the center of the acinus strongly PAS-positive material. I wonder if Dr. Laqueur has seen anything similar to that.

(Dr. Laqueur) I am not prepared to answer the question as to whether Crooke's changes represent hyperactivity or hypoactivity in the pituitary body. I hope I will be able to do so 3 months from now. We have completed a prolonged series of injections of cortisone in dogs. Two of these animals have been killed and the hypophyses have been divided into halves, one half is preserved for histologic study, and the other half is being preserved for bioassay for ACTH, which is in the process, but now I cannot answer the question. It has been suggested by others that the changes are somewhat related to storage phenomena.

I am glad Dr. McManus brought up his question. I am aware that beta granules are not synonymous with basophilia; the latter can be abolished by proper enzymatic digestion while the staining reaction of the granules with anilin blue or PAS is maintained. I am sorry I slipped on that in the paper.

As far as colloid formation is concerned, I am very much interested in that comment because observations like those reported here have given me the stimulus to study colloid formation in the hypophysis. It is true, particularly in thyroidectomized dogs, that large amounts of colloid may be found in the anterior lobe and, in the patients with clinical myxedema, I have shown you the most marked changes we have noted.

OBSERVATIONS ON THE RÔLE OF THE ADRENAL GLAND IN THE PATHOGENESIS OF CARDIAC VALVULITIS, NECROTIZING ARTERITIS, AND GLOMERULONEPHRITIS PRODUCED IN RABBITS BY FOREIGN PROTEINS: EFFECT OF ACTH, CORTISONE, AND COLD ON THE LESIONS. Robert H. More and (by invitation) Sidney D. Kobernick, Montreal, Que.

Abstract. In an effort to evaluate the rôle of the adrenal gland in the pathogenesis of lesions of the heart, arteries, and kidneys of rabbits sensitized to massive doses of foreign serum proteins, three experiments were performed. In the first, rabbits with their backs shaved were exposed to a temperature of 0° to 20°C. throughout the duration of the experiment. Ten cc. of horse serum per kg. was administered intravenously on the 4th and 18th days. In the second, the treatment was similar except that the animals were given 20 mg. of ACTH (lots 77S and 74AX, Armour) per day in two divided doses for 3 days prior to and throughout the exposure to cold. In the third experiment, two groups of rabbits were given two intramuscular injections of 1 gm. of bovine gamma globulin per kg. in Freund's adjuvant 9 days apart. One of these groups also received 20 mg. per day of cortisone in two divided doses throughout the experiment. The organs were weighed at autopsy and compared to the carcass weight (body weight minus gastro-intestinal tract).

Typical cardiac valvulitis, necrotizing arteritis, and glomerulonephritis occurred in the groups injected with horse serum at both room temperature and in the cold. Similar but less severe lesions were seen in smaller incidence in the animals receiving ACTH, but the difference in incidence was not statistically significant. Both groups receiving bovine gamma globulin in Freund's adjuvant showed glomerulonephritis, but the cortisone-treated had a smaller, but not statistically different, incidence. The lesions in the cortisone group were also of milder intensity. Valvulitis and arteritis did not appear in these groups.

The organ to carcass weight ratios of animals that lost body weight did not reflect the organ weights. In those animals that survived the experiment and gained weight, the following trends were noted: The adrenal weight appeared to be slightly increased in animals exposed to cold or given ACTH, but slightly decreased in those treated with cortisone. The pituitary weight was slightly increased in all the experimental groups other than the cortisone group. This slight increase in pituitary weight was most evident in the ACTH treated group. Decrease of the thymus weight was observed in all of the treated groups. Animals dying spontaneously showed no constant change in pituitary or adrenal weight, but had small thymuses. No correlation was demonstrated between the changes in adrenal, pituitary, or thymus weights and the occurrence of lesions.

In all animals injected with foreign proteins, precipitating antibodies were demonstrated in the blood by ring tests and skin sensitivity of the Arthus type was present, even in the animals receiving ACTH or cortisone; but the skin reactivity appeared to be less intense under hormone treatment.

The weight changes in adrenal gland, thymus, and pituitary body show that "stress" was produced in our experiments, but there was no clear correlation between this phenomenon and the occurrence of lesions. Moreover, the Arthus type of skin sensitivity, precipitins, and tissue lesions can develop under treatment with ACTH or cortisone.

Discussion

(Dr. Russell L. Holman, New Orleans, La.) We have been using another method of producing necrotizing arteritis, namely, feeding our standard high fat diet for 8 weeks or longer and then damaging the kidneys, as by bilateral nephrectomy, and under these conditions we can regularly produce necrotizing arteritis in dogs that has most if not all the features of the lesions of human "collagen diseases." We were interested in the effect of cortisone on these lesions. Cortisone in doses of around 5 mg. per kg., given for several weeks along with the diet, definitely prevented the lesions, whereas a smaller dose, less than 1 mg. per kg., failed to do so. It seems to me we are dealing with two separate and distinct problems here, and I have had difficulty in my own mind in trying to reconcile the pathogenesis of these lesions and the protective action of cortisone and/or ACTH. My own feeling at this time, based on a very limited number of experiments, is that cortisone seems to "harden," if I may use that word, the ground or cement substance, thus denying access to the toxic component of the diet which stirs up the imbalance in collagen or ground and cement substances. It is difficult to discuss this in a sentence or two, but I think cortisone denies access of the toxic substance. I wonder in these particular experiments whether Dr. More has any ideas as to the mechanism by which foreign protein actually caused damage, and whether cortisone can, by hardening the ground or cement substance, deny access to the toxic component, such as that involved in an antigen-antibody reaction. My impression is that these reactions have not been stopped in any way by antihistamines, so I am not surprised to find that the adrenal-pituitary mechanism does not have anything to do with the actual pathogenesis of the lesion. I wonder what interpretation Dr. More places on these findings.

(Dr. More) I think that anything we have to say about the basic pathogenesis of these lesions is still quite speculative. The one fact that can be correlated with the lesions we have described is the state of hypersensitivity; it is always present. In experiments where inhibition of foreign serum lesions has resulted from administration of ACTH or cortisone there has not been demonstrated a clear-cut diminution of the hypersensitive state as manifested either by the level of circulating antibodies or the degree of skin sensitivity. While there is no incontrovertible proof that cortisone does diminish the degree of hypersensitivity to antigens, there is good evidence that, at the level of tissue reactions in hypersensitivity, cortisone may depress the reaction of tissues as shown in the decreased rate of repair of traumatic wounds. It

is impossible, therefore, to state whether cortisone or ACTH suppress the development of foreign serum lesions by some effect on the state of hypersensitivity or by some other effect on tissue reaction. Furthermore, the inhibition of lesions by these hormones does not give any clue to whether the hypersensitive state is important in the pathogenesis of the lesion.

A Histopathologic Study of the Involution of Rheumatoid Nodules Treated with Cortisone and of Spontaneous Involution.* Robert Fienberg and (by invitation) Francis L. Colpoys, Framingham, Mass.

Abstract. A 61-year-old white male with severe rheumatoid arthritis of 9 years' duration had eleven scleral rheumatoid nodules (scleromalacia perforans) of the right eye and a subcutaneous rheumatoid nodule in the right olecranon area. The left eye was completely atrophic with no rheumatoid nodules present. The patient received 6.6 gm. of cortisone in 39 days. One scleral nodule was removed 2 months before the beginning of cortisone therapy, and subsequently individual scleral nodules were removed on the 14th, 24th, and 31st days after the beginning of therapy. Another scleral nodule was removed on the 32nd day following cessation of therapy. The nodule removed before therapy was begun showed foci of necrosis surrounded by well polarized palisaded cells. A study of the treated nodules revealed progressive involutionary changes among the palisaded cells such as multinucleated giant cell formation, disturbances of the polarity, increased amounts of cytoplasm, and vacuolization of cytoplasm. Condensation of collagen and central cystic degeneration were seen also. One-half of the subcutaneous nodule in the right olecranon area was removed before, and the remaining half 31 days after, the beginning of cortisone therapy. Foci of necrosis with moderately polarized palisaded cells were noted in the pre-treatment half, while none were seen in the post-treatment half. The patient expired 7 weeks after the cessation of therapy, and active rheumatoid nodules were found in the right eye and in the pleuropericardial tissues. The nodules in the right eye did not have the extensive involutionary changes found in the scleral nodules removed while the patient was receiving cortisone.

Eight rheumatoid nodules obtained from patients who were not treated with cortisone revealed similar involutionary changes but the stages of involution were poorly defined and thus difficult to recognize. Lipoid stains of rheumatoid nodules not treated with cortisone demonstrated the presence of lipoid within the cytoplasm of the palisaded cells. This lipoid probably included neutral fat and phospholipids. The deposition of cholesterol was a late manifestation.

It is concluded that cortisone induces an acceleration of involutionary changes which normally occur in rheumatoid nodules not treated with cortisone and hastens regression by preventing the occurrence of further activity.

Discussion

(Dr. Paul Klemperer, New York, N.Y.) Apropos of this presentation, I should like to refer to some findings in human material regarding changes after treatment. I have so far only 3 cases of systemic lupus which have been treated—2 for so short a time that one cannot draw any conclusions. In these cases the typical histologic lesions were present. The last case, autopsied 2 weeks ago, has been treated for a year and a half. During the life of the patient LE cells have been found in the bone marrow and at autopsy the tissue lesions were perfectly typical and identical with those seen in the previous period of non-treatment. I would like to remark, what you probably all know, that sarcoidosis treated with cortisone shows most remarkable and most dramatic changes. Within a period of 2 to 5 weeks— my observations are only for 5 weeks—the epithelioid cell tubercles are transformed into hyaline nodules

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

which in the untreated cases are seen only after months or years. This hyaline material seems to me in the light microscope to be collagen.

(Dr. Fienberg) At the post-mortem examination it was interesting that only the old lesions and active lesions were found. None of the progressive changes that were seen in the treated nodules was noted, and there was much collagen deposition present.

THE EFFECT OF CORTISONE ON EXPERIMENTAL GLOMERULONEPHRITIS. Chester R. McLean (by invitation), John D. Hamilton, and John D. L. Fitzgerald (by invitation), Kingston and Toronto, Ont.

Abstract. Lesions of generalized necrotizing arteritis and cardiac valvulitis may be readily produced in the rabbit by the intravenous injection of one or two large doses of foreign protein. If the animals are subjected to the operation of left nephrectomy before any serum is given, and if the intervals between serum injections are suitably chosen (11 days), a diffuse glomerulonephritis also is produced in a high proportion of the treated animals. Cortisone in a dosage of 25 mg. per day was employed to observe what effect, if any, the hormone would exert on these serum-induced lesions.

The animals of the control group developed arteritis, cardiac valvulitis, and glomerulonephritis in the expected high incidence of 80 per cent. The daily administration of 25 mg. of cortisone during the experimental period caused a complete suppression of arterial and cardiac lesions among the 12 animals of the treated group. The effect of cortisone upon the serum-induced nephritis was difficult to assess since cortisone itself in this relatively high dosage caused renal glomerular damage consisting of dilatation of individual capillary groups and the formation of protein coagula within such dilated capillaries. Over and above these effects, cortisone did appear to depress the incidence and reduce the severity of the endothelial proliferative changes which characterize serum-induced nephritis. Among the animals of the control group a progressive rise in serum globulin was observed during the experiment. The mean increase for the group was 125 per cent after 18 days of treatment. The administration of cortisone completely inhibited this progressive rise in serum globulin among the animals of the treated group. The failure to develop a high serum globulin among the animals receiving cortisone was probably significantly related to the lack of demonstrable serum precipitin, which was also characteristic of the animals in this group.

Discussion

(Dr. William E. Ehrich, Philadelphia, Pa.) Drs. Forman, Seifter, and I have an exhibit below to show that horse serum nephritis differs from anti-kidney serum nephritis in that there is no endothelial proliferation, but the reaction is one of the intercapillary mesenchyma. Intravenous injection of foreign protein causes serum disease, *i.e.*, widespread involvement of the mesenchyma. In the glomerulus the only true mesenchyma is contained in the intercapillary space. We have found that this intercapillary nephritis is a diffuse disease, affecting all glomeruli; it may or may not be associated with focal extracapillary nephritis, *i.e.*, exudation into the capsular space of fibrinoid material and subsequent organization of same by epithelium. Cortisone in the doses used by us depressed intercapillary nephritis and prevented periarteritis, but it did not prevent extracapillary nephritis, *i.e.*, exudation into the capsular space.

As to the rationale of this treatment it seems that there are a good many reasons why cortisone should act: (1) It is known that it interferes with antibody production through destruction of antibody-forming cells; (2) it enhances catabolism of protein, *i.e.*, degradation of antigen and antibody; (3) it interferes with the permeability of such membranes as are composed of mesenchyma, *i.e.*, ground substance, as was

clearly demonstrated by Dr. Seifter at Philadelphia. The latter effect may be one upon hyaluronic acid or hyaluronidase or both. We know that there are certain anti-hyaluronidases such as the non-specific serum globulins, the specific anti-hyaluronidase antibodies, and heparin, all of which interfere with hyaluronidase activity. We know that hyperglobulinemia, the presence of anti-hyaluronidase antibodies, and hyperheparinemia are frequent features of the so-called collagen diseases. It appears that there are many different ways in which cortisone may act in these diseases.

(Brig. Gen. Elbert DeCoursey, Washington, D.C.) In those animals given cortisone only, was there dilatation of the glomerular capillaries and hematuria?

(Dr. McLean) I do not think I can add anything to Dr. Ehrich's comprehensive remarks.

In reply to General DeCoursey's question, proteinuria did occur in the cortisone-treated animals, all of which exhibited dilatation of the glomerular capillaries, but it occurred whether we treated with cortisone only, or cortisone plus serum. We did not examine the urine for red cells.

EFFECT OF VARIOUS ENDOCRINE GLANDS ON NEPHROTOXIC RENAL DISEASE IN RATS. Walter Heymann (by invitation) and Donald B. Hackel (by invitation), Cleveland, Ohio.

Abstract. Nephrotoxic renal disease in rats was produced by the intravenous injection of anti-rat kidney serum obtained from rabbits. Studies were carried out after hypophysectomy, adrenalectomy, thyroidectomy, oophorectomy, and orchiectomy had been performed prior to and after renal disease had been induced. The effect of pancreatic injury was studied in rats that had been made diabetic by the intravenous injection of alloxan. Quantitative studies of the proteinuria, blood pressure and chemical characteristics of the blood, and histologic examinations of the kidneys were completed in 61 nephrotic rats. Hypophysectomy and adrenalectomy yielded similar results with the degree of proteinuria, of ascites, and of edema, being definitely decreased in these animals. Thyroidectomy, oophorectomy, and orchiectomy were without definite influence. Alloxan diabetes did not aggravate the nephrotoxic disease in spite of the presence of renal disease initially associated with this type of experimental diabetes. It even seemed to take a milder course in some of the rats. None of these endocrine glands had any effect on the hyperlipemia that is regularly associated with this condition.

Discussion

(Dr. William E. Ehrich, Philadelphia, Pa.) Dr. Forman and I are greatly impressed with the work of Dr. Heymann. He was the first to show that the renal disease caused by large doses of a potent anti-kidney serum is not a nephritis, but a lipoid nephrosis. Dr. Forman and I have an exhibit below to show that with large doses of a potent serum the lesions in the kidney are entirely degenerative. There is infiltration of the basement membrane with plasma components leading to thickening of same without any proliferation at all. However, if we gave lower doses of a potent anti-kidney serum, or a less potent serum, then we found proliferative changes, *i.e.*, proliferation of the endothelial cells and to a lesser extent of the epithelium, that is, intracapillary nephritis. Both the degenerative disease and the proliferative nephritis occur in man. Lipoid nephrosis in man is not necessarily associated with any nephritis at any stage. We thus are back at the old concept that lipoid nephrosis is a degenerative disease and thus differs from true nephritis.

A STUDY OF THE EFFECT OF ADRENAL CORTICAL EXTRACT AND PHENAZOLINE HYDROCHLORIDE ON EXPERIMENTALLY INDUCED PERIARTERITIS NODOSA. William R. Platt, Camden, N.J., and Philadelphia, Pa.

Abstract. Periarteritis nodosa, a representative of the collagen-vascular group of diseases, has been shown previously to result, both in the human and in the experi-

mental animal, from anaphylactic hypersensitivity. Recent reports in the literature have been concerned with the use of isolated components of the adrenal cortical hormone in the treatment of the collagen-vascular diseases as well as in serum sickness and drug hypersensitivity. These, and the dramatic clinical response of many disease entities of probable allergic etiology to therapy with the antihistaminase drugs, made it desirable to ascertain whether the entire adrenal cortical hormone or a specific crystalline antihistaminase (phenazoline hydrochloride) would affect the histologic development of the lesions of one member of the collagen-vascular group now definitely known to be producible by anaphylactic hypersensitivity (Rich-Gregory technic), *i.e.*, periarteritis nodosa.

Accordingly, 24 rabbits of both sexes and several colors were divided into two groups. The first group consisted of three subdivisions of 5 animals each (1, controls —horse serum alone; 2, horse serum plus phenazoline hydrochloride subcutaneously; 3, horse serum plus adrenal cortical extract intramuscularly). The second group also consisted of three subdivisions of 3 animals each, all the animals of which received sulfadiazine prior to and concurrently with the experiment. In addition, the members of the subdivisions of the second group received sera (1, horse serum; 2, horse serum plus phenazoline hydrochloride; 3, horse serum plus adrenal cortical extract) in the same manner as did group I. Observations were made throughout the experiment as to weight, body temperature, and untoward reactions. The animals were then sacrificed at varying periods throughout the experiment and gross and microscopic studies made on all organs.

The microscopic results showed very little morphologic variation in the hypersensitive reactions as observed in the skin, lungs, liver, bone marrow, and vascular system. Therefore, while it would appear that an adrenal cortical extract containing all three major constituents—the N–androgenic factor, the S–sugar factor (cortisone), and DOCA (the sodium retention factor)—and an antihistaminase (phenazoline hydrochloride) exerted no decided suppressive influence upon the development of the lesions, this interpretation must be regarded as only tentative in view of the well known and unpredictable differences in individual susceptibility to the development of periarteritis nodosa and other types of hypersensitive reactions in the sensitized human being and experimental animal.

Discussion

(Dr. Valy Menkin, Philadelphia, Pa.) These experiments are extremely interesting. It is very much the same type of reaction which we observed 11 years ago. I would like to know what the effect of ACTH or cortisone was on these animals.

(Dr. Bram Rose, Montreal, Que.) It should be pointed out that the release of histamine by antigen-antibody mechanisms is the result and not the cause of cell stimulation or damage. It is rather unlikely, therefore, that the experimental lesions described by Dr. Platt might be prevented by the administration of an antihistamine compound.

(Dr. Platt) As to what the effects of ACTH would be on these animals, I can only state that at the time these experiments were planned in November, 1949, ACTH and cortisone were not available to everyone who wished to do experiments of this type. Furthermore, I also wanted to see what the histopathologic effects would be of a preparation containing a combination of adrenal cortical steroids.

With reference to histamine production in allergically induced periarteritis nodosa and the effects of antihistaminase on this experimentally produced collagen vascular disease, recent work has shown that cortisone seems to produce the following alterations in histamine metabolism: increased excretion of histidine, decreased formation of histamine, stimulation of histaminase production, and an increased breakdown of histamine by this histaminase-enzyme system. As I have reported during this presentation, phenazoline hydrochloride (an antihistaminase) had no effect whatsoever

on the periarteritic reaction; however, the adrenal cortical extract preparation seemed to suppress the inflammatory reaction, *per se,* which was observed so constantly in the control animals of this mesenchymal degenerative disease.

ENHANCEMENT OF SUSCEPTIBILITY TO EXPERIMENTAL POLIOMYELITIS BY MEANS OF CORTISONE. Gregory Shwartzman, New York, N.Y.

Abstract. ACTH and cortisone in combination, or cortisone alone, produced a marked acceleration of poliomyelitis infection (strain MEF1) in mice, and an extraordinary enhancement of susceptibility to this infection in hamsters, giving rise to a violent and uniformly fatal disease. There was a significant increase in the titer of the virus in the brain of cortisone-treated hamsters. The effect of cortisone was clearly related to the dose injected. One intramuscular injection of 2 mg. 18 hours prior to the intracerebral injection of the virus was sufficient to produce a significant enhancement in the hamster. Mice and hamsters injected intramuscularly four times with 5 mg. of cortisone at 18, 4, and 19 hour intervals and inoculated with the virus intraperitoneally shortly after the second injection of cortisone showed a high mortality with about one-third of the animals developing typical paralysis following an incubation period of 4 to 17 days. The virus was recovered from the brain of animals paralyzed on the 5th and 17th days. All control mice and hamsters receiving no cortisone remained well following intraperitoneal inoculation of the virus. Large doses of ACTH injected repeatedly, DCA, progesterone, and diethylstilbestrol failed to modify the susceptibility of the animals to the infection.

OBSERVATIONS ON THE EFFECT OF SECRETIONS OF THE ADRENAL CORTEX ON INFLAMMATION. Thomas F. Dougherty (by invitation), Salt Lake City, Utah.

Abstract. There are two theories concerning the etiology of mesenchymal diseases. One theory suggests that alterations in the connective tissue are related primarily to an imbalance in the secretions of the adrenal cortex. Another point of view is that the same connective tissue diseases are a manifestation of the hypersensitive state. A third suggestion is presented here which is briefly stated as follows: The basic potentiality for the production of alterations in the connective tissue lies in the antigen-antibody union. The result of this union is the production of inflamed connective tissue which, in turn, provides the pathologic alterations varying according to the extent, duration, and anatomical site of the allergic process. It is postulated that secretions of the adrenal cortex actually modify the extent of the inflammatory process resulting from antigen-antibody union in the tissues. Reduced concentration of C-11 oxysteroids in the tissues enhances the degree of allergic manifestations, whereas increased concentrations diminish this type of inflammation. The effect of cortisone is exerted not by inhibiting the antigen-antibody union but by decreasing either the concentration or diminishing the production of those substances, such as histamine or other agents, which in turn are responsible for the phenomena of inflammation.

According to this point of view, then, treatment of diseases which are primarily allergic in etiology with ACTH and cortisone ameliorates the symptoms of such allergic diseases but may not fundamentally remove the factors responsible for the production of altered connective tissue. Although secretions of the adrenal cortex may possibly be concerned with the production of antibodies, this rôle of the adrenal cortex is not considered fundamental to the problem of the action of C-11 oxysteroids in modifying the results of allergy.

This point of view evolved from interpretation of a large number of experiments concerned with a quantitative study of the rôle of various adrenal cortical steroids in protection against anaphylactic shock and local inflammatory phenomena produced by topical application of antigen to sensitized animals. Changes in the tissues and organs of cortisone-treated animals have been compared in a highly quantitative

fashion with those of intact and adrenalectomized animals. The results of these investigations were treated in detail. Further investigations which have been performed as a consequence of the development of this theory include the relation of endocrine imbalance to the development and extensiveness of inflammation induced by allergy. A method has been evolved by which the concentration of secretions of the adrenal cortex in tissues may be evaluated in such a manner that the capacity of the individual to resist various degrees of stress produced by allergens may be predicted.

Discussion

(Dr. Valy Menkin, Philadelphia, Pa.) These observations are very interesting. They substantiate our original observations of 1940 and 1942. I am interested to know whether it is only a local effect, because recently we have shown that if we inject large doses of ACTH intravenously in rabbits we get also the same suppressing effect by the systemic route on the local inflammatory reaction.

(Dr. Bram Rose, Montreal, Que.) I would like to ask Dr. Dougherty how he reconciles these results with those found in man. For example, ACTH or cortisone are capable of inhibiting the delayed type of skin reaction following the intradermal injection of a bacterial vaccine. This reaction does not appear to be due to histamine release nor is it inhibited by the previous administration of an antihistamine compound. Again, the immediate skin reaction, whether due to the injection of ragweed extract into the skin of a ragweed sensitive subject, or produced by the injection of histamine itself, is not inhibited by ACTH or cortisone. Both of these latter reactions are associated with histamine release locally, and can be inhibited by the previous administration of antihistamine compounds. They would appear to support the contention that ACTH or cortisone does not exert any direct action on the effects of histamine.

(Dr. Dougherty) Your data, Dr. Menkin, are most interesting and I must confess that it leaves me at a loss to attempt any explanation of the direct effect of ACTH which you have described. I wish to make note of the fact that Dr. Menkin was the first person to demonstrate that adrenocortical hormones could inhibit the inflammatory process.

With regard to Dr. Rose's question, I believe that to prevent inflammation produced by hypersensitivity, it is essential that a large amount of the hormone must be present in the tissue previous to subjection of the hypersensitive individual to the homologous antigen. Certainly our work has demonstrated that this is the case. Therefore we are now convinced that varying inflammatory stimuli produce varying requirements for amounts of hormone which will diminish the inflammatory response.

MORPHOLOGY OF THE ADRENAL GLAND IN SYSTEMIC DISEASE. Norman Zamcheck (by invitation), Boston, Mass.

Abstract. It is well known from metabolic studies on humans and animals that the adrenal gland participates in many systemic diseases. Less known, perhaps, is the fact that morphologic changes in the human adrenal cortex also reflect systemic disorders. Sections of adrenal glands were obtained from many necropsies of humans dying of a variety of disorders, including traumatic and burn shock, acute infectious disease (bacterial and viral), chemical poisoning, exposure to extremes of temperature, atomic radiation, starvation, and peritonitis. The following changes were commonly noted: (1) Increase in thickness of the adrenal cortex caused by regeneration of adrenal cortical cells, especially marked in the zona fasciculata, indicated commonly by lengthening and widening of the fasciculata cords and sometimes by mitotic activity. Pseudo-tubule formation of this zone sometimes was seen, and the lumina of the pseudo-tubules contained inflammatory cells, hemorrhage, fibrin, or lipid-filled cells. (2) Alterations in the normal lipid distribution. The outer half of the normal human adrenal cortex is heavily laden with neutral lipid. In overwhelming

systemic disease this lipid regularly diminished in amount, and with increasing severity and duration of illness the lipid tended to disappear from the cortex entirely. In some instances a simultaneous appearance of lipid was noted in the inner cortical layers, normally free of lipid. In illnesses of still longer duration, as well as in convalescence, reappearance of lipid was observed in the outer cortical cells. Occasionally, scattered large or small islands of fat-filled cells persisted in an adrenal cortex otherwise depleted of lipid. (3) Acute inflammation, congestion, and hemorrhage. The above findings sometimes were obscured by massive adrenal hemorrhage which was noted most commonly in overwhelming meningococcus infection, but was found also in other systemic infections. All stages of lesser hemorrhage were found, the zona reticularis appearing more susceptible than either of the other zones or the medulla.

The sequential nature of the changes (1) and (2) was clearly shown in a study of 30 men who died of overwhelming diphtheria, after various intervals of survival. Similar sequences of histologic and of lipid changes have been reproduced in experimental diphtherial intoxication in guinea-pigs. These morphologic findings were not observed in adrenal glands obtained from normal guinea-pigs or from humans dying suddenly of traumatic causes. Hence, they could not be attributed to post-mortem alteration. Although it was not possible to define the chemical or physiologic correlations of these morphologic changes, it is clear that post-mortem examination of the human gland not only provides evidence of overwhelming systemic stress, but also indicates in a general way the duration and severity of the disease. The adrenal glands of patients dying of fulminant systemic disease are readily differentiated morphologically from those of patients dying of chronic illness, as well as from those dying suddenly of non-systemic disease.

Discussion

(Dr. Jacob Werne, Jamaica, N.Y.) I wonder whether Dr. Zamcheck is satisfied that he can distinguish between adrenal glands coming from normal persons dying unexpectedly and those dying after fulminating disease. The impression that I have from my own material is that the fatal disease must run a sufficiently long course to evoke a reaction which will permit labelling the adrenal gland as abnormal after microscopic study. I also wonder how long a period of trauma Dr. Zamcheck has found necessary to produce microscopic adrenal abnormalities in his material.

(Dr. Joshua L. Edwards, Boston, Mass.) I would like to know if there were any other inflammatory changes.

(Dr. Jesse L. Carr, San Francisco, Calif.) In Dr. Zamcheck's observations were there cases in which the stress was purely emotional?

(Dr. Zamcheck) In answer to the first question, it is clear that these changes are not seen in adrenal glands obtained from those who die immediately of traumatic causes. In fact, such cases provide the only normal adrenal glands I have seen. The earliest recognizable adrenal changes occur in about 15 hours. These, however, are not of the types which I have described this morning. The most striking changes of the "pseudo-tubular" type occurred in persons who died of diphtheria of 1 week's duration or longer. You will recall the first slide which was obtained from the adrenal gland of a patient who died of diphtheria of 3 days' duration; histologically, none of the "pseudo-tubular" lesions were seen at this time, and almost no alteration in lipid distribution. Patients dying of trauma who survived for longer periods would show varying degrees of the changes described today. Cases with shock and overwhelming infection appeared to have much more striking "pseudo-tubular" lesions than patients dying of trauma without overwhelming infection. Severely burned patients, on the other hand, regularly showed a marked degree of these changes. The limited time available precluded review of the many other changes which can occur in the adrenal cortex. These include inflammatory changes, thrombosis of sinusoids, fibrosis, ne-

crosis and hemorrhage, to mention a few. Hemorrhage, as you know, is common in overwhelming meningococcus infection.

In answer to Dr. Carr's question, I have no material obtained from cases in which emotional stress clearly predominated.

I am delighted that Dr. Gruenwald confirmed the presence of these findings in the adrenal glands of infants. I have also found changes of these types in children who died of diphtheria.

MECHANISM OF ACTION OF ACTH AND CORTISONE. George W. Thorn,* Boston, Mass.

THE RÔLE OF THE ADRENAL GLANDS IN HYPERTENSION. Georges M. C. Masson (by invitation), Cleveland, Ohio.

Abstract. In rats, renal and hormonal hypertension result in similar vascular lesions. The adrenal cortex in renal hypertensive rats shows hyperplasia of the zona glomerulosa. This zona is the presumptive source of salt-retaining "desoxycorticosteroids." It is irresponsive to ACTH. Its activity depends largely on electrolyte (Na and K) concentrations.

Renin has been found to cause hypertrophy of the zona glomerulosa of rats. Renin is known to cause sodium diuresis. Hypertrophy of the zona also is found in renal hypertension, where it can be attributed to the action of renin. In both cases the glomerulosal hypertrophy can be considered a homeostatic response to renin-induced sodium loss in which the glomerulosa secretes increased amounts of desoxycorticosteroids. Alternatively, assuming that there is only one major cortical hormone, sodium loss would stimulate the secretion and sodium-retaining action of such a hormone. In this connection, compound E (cortisone) alone does not, in our hands, induce hypertensive disease and compound "S" is relatively inert.

Under appropriate conditions, cortical hormones, of which desoxycorticosterone is a synthetic prototype, act to increase arterial pressure and to cause nephrosclerosis. In renal hypertension the pressor effect of glomerulosal steroids would then add to that caused by angiotonin, while progressive nephrosclerotic activity would maintain liberation of small amounts of renin. Thus, in the rat, renal hypertension due to Page's silk perinephritis can be viewed as a self-perpetuating vicious cycle of renal and adrenal interplay. The net effect on sodium metabolism might be a maintenance of nearly normal serum electrolyte concentrations or, as Laramore and Grollman have shown in renal hypertension, a pattern of serum and tissue electrolytes similar to that elicited by desoxycorticosterone overdosage. From the clinical point of view the concept accords with the tendency of many patients with hypertension to retain sodium unduly and to respond to sodium restriction by a decrease in arterial pressures.

In summary, we propose a concept of renal hypertension which would view it at its onset as due to hyperactivity of the renal pressor system, and later, when secondary adrenal glomerulosal hypersecretion begins, as a vicious cycle of renal and adrenal pressor activity. Some of the inconsistencies of other hypotheses are thereby resolved. The status of the adrenal cortex as an accessory to the fact of renal hypertension is also altered to a position more consistent with its importance in the maintenance of normal and of increased levels of arterial pressure.

ADRENAL CORTICAL AND MEDULLARY CHANGES IN EXPERIMENTAL RENAL HYPERTENSION. Lelland J. Rather, San Francisco, Calif.

Abstract. In hypertension in the male albino rat, induced by the removal of one kidney and constriction of the other, the adrenal weight increases in direct proportion to the heart weight. Measurements of cortical/medullary volume ratios show that while the greater part of the weight increase of the whole gland is due to cortical growth,

* By special invitation of the Council.

there is a greater relative degree of medullary growth. Thus while the cortex may triple, the medulla may quintuple, its weight. In these enlarged adrenal glands the mean medullary nuclear volume may be approximately doubled while that of the cortical fasciculata nuclei is increased by approximately 50 per cent. Studies of nuclear/cytoplasmic ratios indicate that cell hypertrophy alone may account for the cortical growth, while in the medulla there is hyperplasia as well. In the adrenal glands of those rats having the largest hearts and with tail systolic pressures at times in excess of 200 mm. of Hg, hyaline droplets are found in paraffin sections within cells of the inner cortical zones, particularly in the fasciculata. These are surrounded by a clear zone in the cytoplasm, are numerous, and may be larger than the cell nucleus. They react with leukofuchsin after periodic acid oxidation. The cells which contain them have large nuclei and nucleoli indicative of increased or, at least, active function. In general, there is no depletion of lipid in these adrenal glands. The findings here presented are considered to be evidence for the specific participation of the adrenal gland in experimental renal hypertension and, if the premise that increased growth means increased function be accepted, that the extent of the adrenal participation is dependent on the severity of the hypertension, especially as this is reflected in the degree of cardiac hypertrophy. The findings might be interpreted by relating the hypertension and cardiac hypertrophy to hypertensin formation, and the adrenal hypertrophy to a functional demand on that organ in association with an increased utilization of hypertensinogen, since there is some evidence that the adrenal glands control the hepatic production of hypertensinogen. An alternative explanation here proposed is that the adrenal hypertrophy is due to the metabolic demand of increased cardiac work and is a special case of the adrenal hypertrophy which for a long time has been known to occur in association with prolonged muscle work. The changes in cortical/medullary ratio offer some support for this view. There is evidence in the literature that in human hypertension there are changes in the adrenal glands similar to those described here in experimental renal hypertension, suggesting a similarity of morphologic and physiologic controlling mechanisms.

Discussion

(Dr. Herbert C. Stoerk, Rahway, N.J.) From experiments with Dr. Kaunitz we had occasion to study rats which were maintained for a long time on a diet deficient in vitamin A. In many of these rats we have found "hyaline droplets" in the cytoplasm of the adrenal cortical cells of the sort just shown by Dr. Rather. The droplets could be visualized best on staining with PAS or with metachromatic stains. In many of the vitamin A-deficient rats there was pyelitis and pyelonephritis. Since the "hyaline droplet" change was most pronounced in animals with advanced pyelonephritis, I wonder whether renal injury represents the causative common denominator for the changes observed by Dr. Rather and ourselves.

(Dr. Rather) I do not believe that these PAS-positive droplets are specific for hypertension; however, I feel that they do not indicate renal damage either, because I have studied animals with renal damage more severe than that in those I reported here, and have not found them. However, these droplets are often very abundant in human material, and I am surprised that no one has discussed them in detail at this meeting. They can be found in both cortex and medulla and have the histochemical reactions which I have described. Recently I have been studying a series of cases of (pre-cortisone era) disseminated lupus erythematosus and found such droplets in all cases in the medulla and in about half of the cases in the cortex, so I cannot regard them as specific for hypertension. I had thought originally they might indicate ACTH stimulation, but I have not been able to produce them in the rat with relatively large doses of ACTH.

INTIMAL AORTIC LIPOID FOLLOWING CORTISONE AND ACTH THERAPY IN YOUNGER AGE GROUPS. Eugenia M. Etheridge (by invitation) and Cornelia Hoch-Ligeti (by invitation), Charlottesville, Va.

Abstract. The observation of a very large amount of fat in the intima of the aorta of a child dying of leukemia, who had been treated with cortisone, has led to the study of the aorta in other young persons treated with both cortisone and ACTH. Random sections of aortas from formalin-fixed autopsy material of 29 patients from 1 to 20 years of age, who had been treated with either cortisone or ACTH, were compared with sections of aorta from suitable control groups of similar age. No significant differences were found in preparations stained with Weigert's elastica stain, or periodic acid. Hematoxylin and eosin and fat stains, however, showed a marked increase of lipoid in the intima and, in several instances, the media, in the age group 1 to 11 years of those treated with cortisone and ACTH. After 11 years of age the amount of fat in the intima of control cases approximated that of treated cases so closely that no significant difference in the two groups was seen. Since most of the treated cases were suffering from leukemia, the question obviously arises as to whether this disease does not cause nutritional changes in the vessel wall which allow lipoids to be absorbed more readily. At present it seems to us, however, that treatment with cortisone has facilitated the accumulation of fat in the aortas of the younger age group.

Discussion

(Dr. Russell L. Holman, New Orleans, La.) I would like to know if these changes were grossly detectable and if the sections came from comparable portions of aorta. We all know that selected sections may be quite different from random sections, and I wonder if the authors feel their control data on this were adequate. We have recently completed a study of the aortic lesions in 300 consecutive autopsies to give us a base line, a "profile of arteriosclerosis," and we have found that every case beyond the age of 7 had some lesions. The earliest case was 1½ years old, and lesions at even earlier ages have been reported.

(Dr. Chester Solez, Buffalo, N.Y.) Was the muscle noted in these sections, and what did it look like—as though it had been replaced by lipoid material?

(Dr. Otto Saphir, Chicago, Ill.) I would like to know whether the authors think these changes are reversible.

(Dr. Hans Popper, Chicago, Ill.) I would like to ask whether you are correlating the arterial changes with the presence of hypercholesterolemia, as it has been known to occur following ACTH treatment.

(Dr. Etheridge) We did not take sections from particular parts of the aorta in these cases. The sections were taken at random from autopsy jars, and so I cannot say whether or not they were from the same parts of the aorta. The aorta was observed grossly in one case and it had yellow streaks.

There was lipoid in the muscle of the media, but the muscle was not replaced by it.

As to whether the changes are reversible, we cannot tell from this study. Of course, we would like to know that ourselves.

Blood cholesterol was not determined in these cases.

INFLUENCE OF THE ADRENAL CORTEX ON THE METABOLISM OF NORMAL AND MALIGNANT LYMPHOID TISSUE. Abraham White (by invitation), New York, N.Y.

Abstract. Data have been obtained establishing that normal and malignant lymphoid tissues produce significant amounts of serum proteins, and that in the immunized animal, a portion of this production is influenced by the level of function of the pituitary-adrenal cortical endocrine system. The data suggest also that the rate of synthesis of new protein in normal and malignant lymphoid tissue is affected by

pituitary-adrenal cortical secretion. The extent of aerobic glycolysis and of amino acid utilization by malignant lymphoid tissue is also affected by the secretory activity of the adrenal cortex. The additional evidence presented, indicating the influence of pituitary-adrenal cortical secretion upon certain metabolic contributions of lymphoid tissue, emphasizes further the functional integration of the adrenal cortex and lymphoid tissue in processes concerned with resistance.

Discussion

(Dr. H. A. Gordon, Notre Dame, Ind.) I would like to ask Dr. White if I understood him correctly that in the animal experiments he always used splenic tissue. Did he work with other lymphoid tissue, like that from the intestinal tract? From our work at Lobund Institute with germ-free animals, we have reason to believe there are certain differences in the nature of the lymphoid tissue from different sources.

(Dr. White) In answer to Dr. Gordon's question, we have worked with spleen, thymus, and mesenteric lymph nodes in the non-immunized animals. With the mesenteric lymph nodes, the influence of adrenal cortical secretion on the addition of protein to the serum is very similar to that observed with the spleen. In non-immunized animals, we have not worked with tissues other than spleen because when one gives a single intravenous dose of antigen, the only tissue that produces significant amounts of antibody is the spleen. One sees antibody production by mesenteric lymph nodes only if the animal is immunized by repeated injections of antigen during a period of several weeks.

A Labile, Humoral, Immune Principle, Cytotoxic to Homologous Neoplastic Cells; Its Depression in Rats Injected with Cortisone. Herbert C. Stoerk, Rahway, N.J.

Abstract. Recently a labile principle, capable of destroying neoplastic cells, was demonstrated by Kidd to be present in lymphoid cells of mice immune to tumor grafts. The resemblance between the properties of this labile immune principle and the one discovered by Landsteiner and Chase to be capable of transferring the "delayed" (tuberculin) type of hypersensitivity appears most striking. Both of these immune principles are assumed to be absent from the circulation, sessile in lymphoid cells, and dependent on the activities of the live cell. These are properties consistent with the demonstration that the destructive action of cortisone upon lymphoid cells coincides with an inhibition of tuberculin reactivity as well as with a suppression of tumor-immunity. Cortisone was found to depress circulating antibodies of the type causative in the "immediate" type of hypersensitivity. This depression, however, was far from complete and was not sufficient to diminish established hypersensitivity of the anaphylactic or Arthus type. At present, it appears impossible to study directly the effect of cortisone upon the immune principle responsible for delayed hypersensitivity. We have succeeded, however, in studying its influence on the apparently analogous principle which confers "tumor-immunity." Previously it was found that in mice and rats transitory induction of a riboflavin-deficient state causes regression and complete disappearance of lymphosarcoma implants. It was seen also that, following the regression of the tumors, the animals could not be re-inoculated with the same neoplasm. The nature of this tumor-immunity has been investigated further. This immunity was gradually lost over a period of about 1 year and it rapidly diminished when marked loss of normal lymphoid tissue was produced by the administration of cortisone or of desoxypyridoxine. In rats in which lymphosarcoma implants had regressed during riboflavin deficiency, we found, in addition to a labile, cellular immune principle, its humoral counterpart. Plasma and serum from such animals were strongly cytotoxic to lymphosarcoma cells. Such sera lost their activity when they were exposed to 42° C. for 15 minutes. The immune principle was present in the gamma-globulin fraction but absent from albumin of immune sera. Following

separation from the whole serum the cytotoxic principle proved to be stable to heating below coagulating temperatures. The humoral immune principle was found to appear gradually in lymphosarcoma-bearing animals. The activity of the sera was highest soon after regression of the tumors and then gradually declined. In rats whose titers had declined, re-injection with suspensions of lymphosarcoma cells caused a rapid rise of cytotoxic activity of the sera (anamnestic rise). Daily injections of cortisone, within 1 week, caused nearly complete disappearance of the humoral immune principle.

FOCAL ANAPLASIA OF THE FETAL ADRENAL CORTEX. John M. Craig (by invitation) and Benjamin H. Landing, Boston, Mass.

Abstract. Thirty-nine instances of focal anaplasia of the fetal adrenal cortex, occurring as incidental findings in the adrenal glands of newborn infants, are reported. The lesion is characterized by enlarged cells with enlarged, hyperchromatic, irregular nuclei, which often contain inclusions, occurring singly or in clusters in the fetal cortex; the cells show the high fat and ketosteroid content of normal fetal cortical cells. The process shows no apparent relation to the underlying disease, to the cause of death, or to superimposed infections, and appears unrelated to "inclusion disease" or to "hepato-adrenal necrosis with inclusions." All affected glands show evidences of involution of the fetal cortex, and the lesion may represent an atypical involutionary change. The similarity in cell type suggests a relation to the anaplastic form of masculinizing adrenal cortical carcinoma. The age distribution of this tumor in children, and the relation of such tumors to the adjacent cortex, also suggest that this tumor may be of fetal cortical origin.

Discussion

(Dr. Wallace H. Clark, Jr., New Orleans, La.) I should like to ask if any abnormalities were present in the mothers of these infants.

(Dr. Crichton McNeil, Salt Lake City, Utah) We recently had 2 cases of a similar nature in 1-week-old infants. Both of these infants were from diabetic mothers. The mothers survived, but both infants were dead within 1 week after birth. I wonder if Dr. Craig has found any of his patients who fall into that group. We did not consider it carcinoma.

(Dr. Lelland J. Rather, San Francisco, Calif.) I would like to comment on these findings. One very careful observer, the late Dr. Nicholson of Guy's Hospital in London, remarked that in almost every adrenal gland obtained by necropsy, nuclear changes of this type could be seen, and they have been found even in normal subjects. In Germany, Liebegott described the adrenal glands of 8 healthy condemned men, removed 30 minutes after they were hanged, and found occasional nuclei of this character. I have seen these large nuclei, in clusters, very commonly in adults, and I have seen a few in newborn children. In some instances I have carried out nuclear volume measurements and have obtained volume peaks that had an integral relationship to each other, which suggests that these are actually polyploid nuclei. One explanation of their formation would be a failure of division of the cytoplasm after nuclear division, giving rise to a binucleate cell with two diploid nuclei. Formation of a single spindle at a succeeding metaphase, with chromosomal division, would yield two cells with single tetraploid nuclei of approximately twice the nuclear volume of diploids.

(Dr. William H. Sternberg, New Orleans, La.) Dr. James B. Arey, Dr. John Dent, and I have been interested in this subject, and for a number of years have been collecting examples of changes in the adrenal cortex in fetuses and newborn infants similar to those described by Dr. Craig. Our observations have been similar to his. We have no specific suggestions to make as to the significance of these unusual cells, but I would like to comment on a number of findings in our series. We have had a

number of instances of such histologic change occurring in the offspring of both diabetic mothers, and mothers with toxemia of pregnancy. We have seen changes in a considerable series of fetuses and in some of these the histologic change was very extensive. I should also like to mention one instance of newborn twins in which such anaplastic cells were present in the adrenal glands of both offspring.

(Dr. Kornel Terplan, Buffalo, N.Y.) I would like to add a word to the comments by Dr. McNeil and Dr. Sternberg. It might be very interesting to examine the glands, in all cases in which these peculiar changes in the adrenal cortex are found, especially in premature or full-term babies of diabetic mothers. Cell changes very similar to those shown in the adrenal cortex can be demonstrated in the interstitial cells of the testicle. I might add that the weight of the adrenal glands in these cases from diabetic mothers is exorbitant and closely approaches the weight of the kidneys. In one of the cases studied recently in our laboratory, the weight of both adrenal glands was 27 gm., as compared to 30 gm. for both kidneys.

(Dr. James B. Arey, Philadelphia, Pa.) I would like to add that we have seen a comparable lesion on one occasion in a section of ovary from an infant; this specimen was submitted by Dr. Lester Rauer. I would also like to comment on the frequency of this adrenal lesion in infants of diabetic mothers. The lesion has been described in a number of erythroblastotic infants, and I wonder if the hyperplasia of the adrenal glands noted grossly in infants of diabetic mothers and in erythroblastotic infants may be related to similar changes in the fetal cortex.

(Dr. Craig) With regard to the histories, one of our children came from a diabetic mother.

The frequency of erythroblastosis I think probably should be related to the large size, as Dr. Arey suggested, of the adrenal glands, and also perhaps secondarily to the relative anoxemia of the child and the difficulties of transplacental oxygen transport.

I think it is interesting to note that the child of the diabetic mother also had large hyperchromatic cells throughout the islets of Langerhans. Those working on the pathology of diabetes assure me this is quite a common finding in the pancreas of these children.

About this finding in other conditions, we know of the article of Steinbiss on the presence of similar changes in the hyperplastic regenerative nodules in Addison's disease.

We had two sets of twins in our series and, unlike the presentation from the floor, the adrenal glands of neither of the siblings of the affected infants contained the unusual cells.

PATHOLOGIC CHANGES IN THE ADRENAL CORTEX OF ASPHYXIATED NEWBORN INFANTS. Peter Gruenwald, Brooklyn, N.Y.

Abstract. It is well known that many infants dying in utero or shortly after birth show at autopsy various effects of severe anoxia; more recently it has been suggested that some of their lesions indicate the existence of shock. Among these lesions are necrosis and hemorrhage in the adrenal cortex. Necrosis as indicated by disintegration of cells and their nuclei occurs in the deeper layers of the permanent cortex either focally or, in severe cases, becomes confluent so that an almost uninterrupted zone is affected. Severe congestion of the capillaries with atrophy of the cortical cell cords frequently occurs in the central portion of the organ, and occasionally leads to focal hemorrhage with destruction of the normal architecture. These hemorrhages have been mistaken for effects of mechanical trauma.

It appears improbable that these changes are due to direct effects of anoxia, *i.e.*, to oxygen deficiency of the adrenal cortex. More probably they indicate an excessive reaction of the adrenal gland to anoxia and shock. All of the lesions found in these infants are known to occur in the course of the alarm reaction which includes shock.

It is noteworthy that congestion and hemorrhage in the adrenal cortex of asphyxiated infants resemble changes in the Waterhouse-Friderichsen syndrome; the latter has also been considered as a severe alarm reaction. These observations, and others not to be discussed here, suggest that asphyxiated newborn infants might benefit from treatment for shock and insufficiency of the adrenal cortex, in addition to other measures designed to combat anoxia and possibly lesions in other vital organs.

Discussion

(Dr. Israel Davidsohn, Chicago, Ill.) I would like to ask about the relation of this lesion to the type of delivery; how frequently have you observed these lesions after abnormal operative delivery? Furthermore, how long was the interval between the time of death and the autopsy?

(Dr. Gruenwald) It was quite striking that there is frequently a relation to difficult delivery of various types. I did not take time to cite all the instances, but similar histories were found in many cases. One of the severe cases shown here was actually one which, in a statistical survey of anoxic changes, had one of the highest scores of nearly 500 cases, so while I cannot correlate this with any particular type of delivery, I think there is a good correlation with difficult delivery of any type.

In regard to the time interval between death and autopsy, the time was usually not very short, and the question of post-mortem changes has come up; in the very first slide which I showed, the very diffuse change might be that. However, usually there are focal changes and the remainder of the tissue is well preserved; therefore I do not believe that these are post-mortem changes.

I forgot to mention that changes are found in stillborn as well as liveborn infants, whereas some of the other manifestations of shock, *e.g.*, intestinal ulceration, take several hours after birth to develop.

(Dr. Davidsohn) How often are these changes unilateral?

(Dr. Gruenwald) They are seen quite frequently to be more severe on one side than on the other, but I do not remember having seen a perfectly good adrenal gland on one side and a bad one on the other.

PATHOLOGIC LESIONS IN ADRENAL GLANDS OF DOGS AFTER FULMINATING ANOXIA. R. H. Rigdon and (by invitation) H. G. Swann, Galveston, Texas.

Abstract. The process of anoxic death in dogs with respect to circulatory and respiratory failure has been described by one of us (H. G. S.). When dogs are given pure nitrogen to breathe, their respiration and circulation fail within approximately 4 minutes. They may be readily resuscitated by periodic insufflation of the lungs with oxygen combined with extrathoracic cardiac massage, after 6½ minutes of anoxia. After this experience, the dogs remain stuporous and ataxic for many hours. Then they sink into coma and death occurs shortly thereafter. This experimental procedure produces severe cerebral lesions and also degeneration of the adrenal cortex. Animals sacrificed after 18 hours have focal areas of degeneration in the zona fasciculata with a diffuse infiltration of polymorphonuclear leukocytes. The glomerulosa appears normal. This lesion is more marked in those dogs showing the more severe clinical symptoms. Dogs sacrificed 5 days after the period of anoxia show few, if any, pathologic changes in the adrenal gland. Ten dogs have been studied. Only the lesions in the adrenal gland are reported at this time.

Discussion

(Dr. Jacob Werne, Jamaica, N.Y.) Did you examine histologically the adrenal glands of any of the dogs that died immediately after the administration of nitrous oxide? The reason I ask is that in my experience persons dying following nitrous oxide anesthesia or after exposure to sewer gas where the asphyxial mechanism is presumably similar, show no distinctive lesions. I wonder, therefore, whether the

changes described in the adrenal gland are non-specific ones, resulting from the survival of the animals in a state of shock?

(Dr. Tom R. Hamilton, Kansas City, Kans.) I wonder if this apparent coagulative change in the cytoplasm is of the nature of the change seen with cytoplasmic vacuolization in other organs, and which was associated with anoxia in human material by Kritzler and experimentally by Bunting along with Martin and Loevenhart.

(Dr. Jesse L. Carr, San Francisco, Calif.) Up to approximately 1937, nitrous oxide anesthesia was the routine anesthesia for thyroidectomy at the University of California Hospital, and during that time we had 3 cases with adrenal lesions similar to those described from nitrous oxide anesthesia, one of which lived 30 days practically decerebrate before she died. The lesions were not dissimilar to those shown, and I wonder whether Dr. Rigdon observed such things with nitrous oxide.

(Dr. Rigdon) We do not have any observations earlier than 18 hours. We feel that this interval of 18 hours is adequate to account for the pathologic changes we have seen in these sections. I think that the interval between the time of the severe anoxia and the time you examine the tissues is a very important factor.

I am not able to say any more relative to this change in the cytoplasm of the cells. We merely observed it and feel that it is one of the earliest changes to occur in these animals.

I am glad to know that this lesion occurs in humans. I have not had any experience with this in human material.

A Morphologic Study of Adrenal Glands with Correlations of Body Size and Heart Size. Robert O. Holmes (by invitation), Henry D. Moon, and James F. Rinehart, San Francisco, Calif.

Abstract. A morphologic study of the adrenal glands of 200 persons dying from trauma or natural causes was made. There were 129 males and 71 females with an age range of 16 to 89 years. The combined weight of both glands was determined and histologic studies were made. The adrenal weights varied from 3.9 to 23.3 gm. Males had heavier adrenal glands than females. However, when corrections were made for body size, females averaged slightly more adrenal gland tissue per kg. of body weight and per square meter of body surface than did males. The mean weights are shown in Table I.

Table I

Mean Adrenal Weight, Absolute, and Corrected for Body Size *

	Males	Females	Total
Mean adrenal weight observed, gm.	12 ± .28	10.8 ± .33	11.6 ± .21
Mean adrenal weight, gm. per kg. of body weight	0.183 ± .0046	0.207 ± .0081	0.192 ± .0043
Mean adrenal weight, gm. per sq. meter of body surface	6.88 ± .16	7.16 ± .21	6.98 ± .13

* Means are in gm. and are followed by their standard errors.

There was a tendency for persons with enlarged hearts to show an increase in adrenal weight. When these two organs were compared in terms of gm. per square meter of body surface, this relationship was shown by a correlation coefficient (Pearson) of: $r = +.304 \pm .049$.

The weights and histologic characteristics of the adrenal glands of a group showing no cardiac enlargement (heart weights of 175 gm. or less per square meter of body surface) were compared with those of a group with enlarged hearts probably due to hypertension (250 or more gm. per square meter of body surface). Three of

the 4 cortical adenomas in the entire series fell into the large heart group. The adrenal glands of the group with large hearts had a mean weight increase, an increase in the incidence of nodularity of the cortex, more sclerosis of the capsular arterioles, and a general increase in cortical lipoid. The magnitude of these differences is illustrated in Table II.

TABLE II

Comparison of Adrenal Glands of Humans with Normal and Enlarged Hearts

	Males		Females	
	Normal hearts 23	Enlarged hearts 36	Normal hearts 30	Enlarged hearts 13
Mean adrenal weight, gm. per sq. meter of body surface	5.7 ± .22	7.58 ± .29	7.1 ± .3	7.88 ± .49
Percentage with nodular cortices	21%	49%	20%	36%
Percentage with capsular arteriolosclerosis	7%	46%	10%	21%
Percentage with increased lipoid	17%	31%	10%	7%

The adrenal glands with a nodular cortical pattern had a greater mean weight than did those with a normal pattern. The mean heart weight of persons with nodular adrenal glands was also increased. There was a greater percentage of glands with capsular arteriolosclerosis and increased lipoid in the nodular group. The differences are illustrated in Table III.

TABLE III

Comparison of Adrenal Glands of Normal and of Nodular Patterns

	Normal pattern (142)	Nodular pattern (58)
Mean adrenal weight, gm. per sq. meter of body surface	6.7 ± .14	7.56 ± .29
Mean heart weight, gm. per sq. meter of body surface	207.5 ± 4.5	233 ± 6.9
Percentage with capsular arteriolosclerosis	21%	44%
Percentage with increased lipoid	15%	45%

Discussion

(Dr. Chester Solez, Buffalo, N.Y.) Was there any correlation between diabetes and nodular hyperplasia of the adrenal glands? There was a report about 5 years ago by Russi, Blumenthal, and Gray in which they correlated both hypertension and diabetes, and I wonder if you correlated diabetes with cortical hyperplasia.

(Dr. Hans Popper, Chicago, Ill.) Did this nodular hyperplasia involve the central portion of the cortex or only the peripheral part?

(Dr. Albert Segaloff, New Orleans, La.) We have been very much interested in the problem of the adrenal glands in hypertension and have been studying microscopically specimens obtained from patients subjected to sympathectomy. We have found it difficult to find anything which correlates with the degree of hypertension. I wonder whether the picture seen in the adrenal glands here may not be related to the clinical course of the patient, because, at least clinically speaking, patients with a big heart are sick a lot longer before they die and some of these pictures could be due to the fact that the adrenal glands are from chronically ill patients.

(Dr. Tom R. Hamilton, Kansas City, Kans.) In agreement with Dr. Rinehart's

remarks, when we studied nodularity of adrenal glands in cases of hypertension a few years ago, we found that the incidence of cortical nodules approximating 1 cm. in diameter was 2½ times as high in the hypertensive group as in those who had had no known hypertension.

(Dr. Rinehart) I cannot answer the question raised by Dr. Solez regarding diabetes. We do not have clinical data on this group of cases.

In regard to Dr. Popper's question about the central portion of the cortex, I may say that the irregular nodular and hyperplastic change is present in the deeper portions of the gland as well as peripherally.

In response to Dr. Segaloff's comment, I wonder a little about the adequacy of biopsy material for the evaluation of hyperplasia in the adrenal gland. The normal adrenal gland has a good deal of lipid in it and small pieces might not be entirely representative. However, there may be differences between his material and our own. The cases studied by us had not been hospitalized. They had died as a result of trauma or suddenly of natural causes.

FURTHER EVIDENCE BEARING ON THE MECHANISM OF THE RESPONSE OF THE PITUITARY GLAND TO ACUTE STRESS. Paul L. Munson (by invitation) and Roy O. Greep (by invitation), Boston, Mass.

Abstract. The rapidity of the response of the anterior pituitary gland of the rat (under pentobarbital anesthesia) to an intravenous injection of histamine has been estimated by subsequent administration at varying intervals of a uniform dose of adrenal cortical extract of proved inhibitory potency. The occurrence of a pituitary response was established by the decrease in adrenal ascorbic acid concentration 1 hour after stimulation. No inhibitory action remained when the interval between injections was longer than 10 seconds, and inhibition was only partial after intervals of 3 and 5 seconds, respectively. These findings indicate that the reaction time of the pituitary gland under the experimental conditions was of the order of 10 seconds or less. Conformity with the hypothesis that all stimuli which lead to an increased outpouring of ACTH do so by depleting the blood of cortical steroids below a certain threshold, necessitates clearance of steroids from the blood with greater rapidity than appears justified by present evidence.

Natural and synthetic l-epinephrine also were administered intravenously to anesthetized rats, and, unlike histamine, had no detectable effect on the pituitary gland as measured by depletion of adrenal ascorbic acid. Other experiments have provided further evidence that epinephrine is not an obligatory intermediary between histamine and the response of the pituitary gland. Unanesthetized rats were administered histamine or epinephrine intraperitoneally at dosage levels which have a comparable effect on the adrenal ascorbic acid as observed 60 minutes later. Under these conditions, histamine exerted its effect much more rapidly than epinephrine. The reduction in adrenal ascorbic acid 15 minutes after histamine was highly significant; it was negligible 15 minutes after epinephrine.

Two other stresses, exposure to ether and intraperitoneal administration of purified pressor principle of the posterior pituitary lobe, were like histamine in that they reduced the adrenal ascorbic acid within 15 minutes. A pain stimulus, on the other hand, resembled epinephrine; it reduced the adrenal ascorbic acid in 60 but not in 15 minutes.

The experimental facts presented are not in harmony with either of the two hypotheses considered, which have been advanced to explain the adrenocorticotropic response of the pituitary gland to acute stress. We are not yet prepared to bring forward another explanation.

Discussion

(Dr. Albert Segaloff, New Orleans, La.) There is one thing I would like to point out. I think this is a very beautiful piece of work, but I still do not think we can

throw out the hypothesis of steroid utilization, because from a lot of the experimental work that has been done—true, with estrogens—it only takes one circulation through the liver to reduce the amount by 90 per cent, and I think 10 seconds in a rat is pretty adequate time for hepatic circulation.

(Dr. Munson) I am much interested to hear Dr. Segaloff's comment about the reduction of steroids in the blood of the rat after only one circulation through the liver. The circulation time in the rat is very rapid indeed, a matter of only 6 to 10 seconds. I should like to call attention to the fact that in our experiments there was rather poor inhibition by adrenal cortical extract even when it was given in as short a time as 3 to 5 seconds after administration of histamine.

* * *

SQUAMOUS CARCINOMA OF THE CONJUNCTIVA AND CORNEA OF BOVINES ("CANCER EYE") OF CATTLE. William O. Russell and (by invitation) George S. Loquvam, Houston, Texas.

Abstract. "Cancer eye" is a term that is commonly used for a squamous carcinoma occurring in the ocular mucous membrane of bovines. Specimens of "cancer eye" were obtained for study from Federal Meat Inspectors and had been collected at the time of commercial slaughter. Three hundred and eight eyes were examined grossly and 210 microscopically. These came from 256 cows and 42 steers. The difference between the two sexes was not regarded as significant because cows were slaughtered at an older age than steers. The estimated average age was 5 years. Pigmentation of the skin of the lid was noted as follows: no pigmentation, 34 per cent; partial, 38 per cent; complete, 8 per cent. The degree of pigmentation was not regarded as significant since it corresponded roughly to the cattle population having no, or partial pigmentation of the lid (Hereford breed). The tumor was primary on the cornea, usually at the limbus in 50 per cent of the cases, on the mucous membrane of the lid in 22 per cent, on the nictitating membrane in 17 per cent, and of questionable origin in 11 per cent. Metastasis to the homolateral parotid lymph node was observed in 15 per cent of the cases. It was found that in most instances the development of cancer could be shown to have taken place in a previously existing squamous papilloma. Squamous papilloma was preceded by a plaque of keratomalacia at the limbus. The carcinoma in most instances was a well differentiated squamous type. Of the suggested factors of possible significance in the causation of the tumor, such as absence of pigmentation of the lid, prominence of the eye, the collection of irritating bodies in the tear sac, and living agents (bacteria, viruses, metazoa), virus has the most merit. The evolution of the cancer from a plaque-like thickening of the epithelium to a papilloma that undergoes malignant transformation suggests a virus. The Shope papilloma follows a similar sequence in its development although the two lesions anatomically are not entirely comparable.

Discussion

(Dr. Herbert Z. Lund, Cleveland, Ohio) I should like to ask Dr. Russell if he has followed the development of this lesion from the plaque, to the papilloma, to the invading lesion, or are these isolated eyes which were examined and the idea of the development drawn from the group as a whole? I think there is a difference in interpretation.

(Dr. Béla Halpert, Houston, Texas) Did these tumors occasionally occur bilaterally? The fact that they occur only on one side, if this is the case, would perhaps be against an infectious origin, in which case bilateral occurrence would be more frequent.

(Dr. John H. Rust, Oak Ridge, Tenn.) Does this have any relationship to pink eye, or is it a hereditary or familial carcinoma, as suggested by some workers?

(Dr. Helen Ingleby, Philadelphia, Pa.) Have you any evidence that this disease occurs more frequently in one part of the country than in another?

(Dr. Russell) Regarding the interpretation of the development of the lesion: we found no cancer developing from a carcinoma *in situ*. The smallest carcinomas observed were in all instances associated with a papillary type of change. The eyes having the carcinoma in an advanced stage obviously did not show a papilloma because that lesion was destroyed. The most significant point was finding in the same eye, occurring as separate and distinct lesions, the plaque of thickened epithelium, the squamous papilloma, and a squamous carcinoma arising in a papilloma.

As to the bilateral origin of the cancer: it is reported by ranchers as frequently occurring bilaterally. We have observed the disease in both eyes in 8 animals occurring either as bilateral carcinoma or as cancer associated with papilloma. However, it must be remembered that our material was collected by federal meat inspectors at the time of commercial slaughter. In most instances ranchers were attempting to get the cow or steer by the meat inspector, for which reason only early to moderately advanced lesions were observed. It is not likely they would allow time for the development of lesions in both eyes before selling the animals for slaughter.

A possible causal relationship to pink eye has been suggested by several investigators. Our studies did not show evidence of any type of chronic inflammation in the eye to suggest that infection could have been the inciting factor. If chronic infection was of significance, it would seem that some evidence of infection would still be evident in the eye. Screw-worm has been suggested as a possible inciting agent. We have found screw-worms in a few cases, but the number was entirely too small to be considered significant. No relationship to hereditary familial carcinoma was noted.

In regard to geographic areas of occurrence: It has been suggested in the literature that it occurs in areas having a high actinic stimulation. Such an area would be in the southwestern part of the United States. Our study does not throw light on this question since it was confined to a single geographic area, reportedly of high incidence of cancer eye and having a known high level of actinic stimulation. I should like to point out, however, that one of the original descriptions of the disease at the turn of the century by Leo Loeb, and I might add one of the best and most accurate ones, concerned a herd of cattle in Wyoming. A significant number of cases were reported from a single ranch in the area to suggest an epidemic pattern. This certainly would not be regarded as an area of the country having high actinic stimulation.

THE EFFECTS OF VIRUS THERAPY ON THE MICROSCOPIC STRUCTURE OF HUMAN MELANOMAS. George K. Higgins and George T. Pack, New York, N.Y.

Abstract. At present subcutaneous virus inoculations have been used to treat over 30 patients with malignant melanomas. Clinically, in 6 or more the dermal metastases have decreased in size and become flattened. It is our impression that the development of new metastases was retarded also. At intervals after treatment some of the metastatic cutaneous lesions were removed from 3 patients for microscopic study, the results of which form the text of this presentation.

The degree of change evident on microscopic study varies greatly in different lesions in the same and different individuals. The least effect evident was noted in the lesions present for the longest time. This agrees with the findings of Cox and his co-workers who found that the older leukemic lesions of chickens were less susceptible to virus therapy than the more recent ones. In these cutaneous melanomas, the chief effect appears to be a decreased vascularity. Even though the lesions decrease considerably in size (become flattened), the other changes described below have not been prominent. Newly formed lesions show the most marked effect; there was necrosis and degeneration. In fact, in one specimen the tumor cells had become so disintegrated that the actual nature of the tumor was not evident. It has not been determined at this time whether the more marked regression in the recent lesions resulted from direct action on the tumor cells or on the vascular supply. When these recently formed lesions were removed a month or two following marked regression,

they appeared as pigmented dermal scars. Careful search, however, usually revealed a few recognizable cancer cells. Time is too short to judge the subsequent course of the patients, but, however, the temporary retardation of the cutaneous lesions offers promise in this type of therapy for human cancers.

Discussion

(Dr. Emmerich von Haam, Columbus, Ohio) Will Dr. Higgins tell us more about the type of treatment—what kind it was, and how often it was given?

(Dr. Helen Ingleby, Philadelphia, Pa.) I would like to know whether you have given repeated treatments at intervals, and if so, whether the later treatments were as effective as the first one.

(Dr. Herbert Z. Lund, Cleveland, Ohio) I think it is very difficult to judge improvement in any kind of tumor. Cancer statistics are the worst we have, especially those of melanoma. We have a man in one of our clinics at the University who, from his history, had metastases in 1929 to an inguinal lymph node. In 1939 he did something about it. The nodes and a primary tumor in his foot were excised. A few years later he had metastases to the inguinal lymph nodes of the opposite side, proved by excision and biopsy, and he is still living today. It was probably 20 years ago that metastasis occurred, and he is still "going strong." I do not know what special treatment he had, except excision.

(Dr. Richard C. Taylor, Louisville, Ky.) Has there been any difficulty in treating the patients with rabies vaccine—have there been any untoward effects?

(Dr. J. F. A. McManus, Charlottesville, Va.) I believe with Dr. Lund that there are cases that have metastasized to the lymph nodes and with nothing more than simple excision long periods of survival can occur. Is there any evidence that the virus vaccine has actually gotten into the tumor, besides its apparent regression? If this is a living virus can it be recovered from the lesion, or is there any inclusion which appears, anything resembling the Negri body of the central nervous system?

(Dr. Higgins) As to the type of treatment, at present regular rabies vaccine has been given, and we have given 20 daily doses—nothing special. Repeated series of doses apparently do help; the woman who has had the hepatic lesion regress has had two courses of treatment within a period of 3 months; and even three series of treatments have been given. Of course they are painful.

As to long survival, I agree that there are patients who have had an interval of many years between the removal of an eye and metastases, and we have all seen patients who survived a long time after lymph node metastases from cutaneous lesions, but in all the melanomas that Dr. Pack has seen he has found practically no cases which survived more than 5 years after distant metastases had established themselves.

I do not think I can answer the question as to the untoward effects of virus other than on the tumor. One case did develop some cerebral manifestations. Whether or not this was due to the virus I am not certain.

No studies have been done as yet on finding the virus in the tumor cells. There is no morphologic evidence, that we can see, that the virus is present in the cells themselves. I hope to investigate this in the near future.

DIFFERENTIAL GROWTH OF BLOOD-BORNE METASTATIC TUMORS IN LIVER AND LUNG (EXPERIMENTS WITH RABBIT V-2 CARCINOMA). B. Lucké, C. Breedis, and (by invitation) Z. P. Woo, L. Berwick, and P. Nowell, Philadelphia, Pa.

Abstract. Experiments were designed to test the widely held hypothesis that of the two organs most often involved in the spread of cancer, namely the liver and the lung, the liver affords the more favorable environment for metastatic tumor growth. This hypothesis is largely based upon clinical and post-mortem observations, not on experiment. Comparing the sizes of metastatic cancers in livers and lungs in a series

of 154 human autopsies in which adequate measurements were recorded, the mean size of the largest tumors was seven times greater in the liver than in the lungs. Such analysis is suggestive but, because of the nature of the material, not conclusive.

The hypothesis was therefore tested by means of experiment, using the rabbit V-2 carcinoma. All animals received a suspension of tumor cells in a systemic vein and in either the portal vein or the hepatic artery. The animals were sacrificed at intervals of from 12 to 35 days after inoculation; the liver and lungs were removed, hardened, sliced, and the number and size of the tumors were accurately determined. In the first experiments the concentration of the tumor cell suspension was varied between liver and lung. The number of resulting tumors was roughly proportional to the concentration of the cell suspension, but in all of the animals the mean size of the tumors was greater in the liver than in the lung. In the remaining experiments, any given animal received the same concentration of tumor cells in the liver and the lung. In 14 rabbits the size of tumors in the lung was compared with the size of tumors in the liver resulting from injection via the portal vein. At any stage of growth, the mean size of the liver metastases exceeded the mean size of the lung metastases. In 7 other rabbits the size of tumors in the lung was compared with the size of tumors in the liver resulting from injection via the hepatic artery. The same relation as stated above was found. In other words, the liver tumors always exceeded the lung tumors in size, no matter what the route of introduction. The rate of increase in tumor size differed in the two organs. Twelve days after inoculation the liver tumors were about five times greater in actual volume than the lung tumors, and 35 days after inoculation the liver tumors were from 100 to 200 times greater in size. The data given apply to the mean size of tumors. Since the number of tumors was usually greater in the lung than in the liver, the total volume of tumor mass was computed. This showed the same trend as did the mean size.

It is concluded that the experiments with rabbit V-2 carcinoma support the hypothesis that the liver is a more favorable environment for growth of metastatic cancer than is the lung.

Discussion

(Dr. Otto Saphir, Chicago, Ill.) I would like to ask if the authors have any explanation for these very interesting findings. Perhaps it may be that in the lung, when doing autopsies on patients with tumors, one often finds tumor cells in branches of the pulmonary artery in various stages of organization in the absence of metastases. Of course, it is not the tumor cells which become organized, but tumor cell emboli apparently cause the formation of hyaline thrombi. I do not know whether such changes do or do not occur in the branches of the hepatic veins or the portal veins, but if not found in these vessels, I wonder if this may not constitute the explanation for the finding of large tumors in the liver and small tumors in the lung.

(Dr. Gert L. Laqueur, Bethesda, Md.) Are there any observations available with choriocarcinoma?

(Dr. Breedis) In regard to the first question, we believe that differences between lung and liver with respect to differential growth of metastatic tumors are perhaps due to two main factors: (a) differences in the chemical and physical constitution of these two organs, and (b) difference in their temperature. A distinction must be made between two processes: establishment of tumor emboli and the growth of established tumors. It is with the second of these that we are here concerned. Organization of thrombotic material around tumor emboli may play an important part in the establishment of tumor emboli but it can play no part once the emboli have gained a foothold and have started to grow.

In reply to the second question, we have done nothing with choriocarcinoma.

ADENOCARCINOMA OF THE BLADDER. F. K. Mostofi, R. V. Thomson, and A. L. Dean, Jr. (all by invitation), Washington, D.C.

Abstract. The finding of a glandular neoplasm in a bladder biopsy poses a number of difficult questions for both the pathologist and the clinician. In order to clarify some of these problems a study was undertaken of a group of 55 cases coded as adenocarcinoma of the bladder in the files of the Armed Forces Institute of Pathology. The selection was limited only to cases in which there were clinical symptoms of a primary disease of bladder. Most of these tumors are found to be of bladder or urachal origin and not metastatic. Evidence derived from this study suggests that mucinous and glandular potentialities of the transitional epithelium of bladder may play a rôle in the histogenesis of these tumors.

Discussion

(Dr. Béla Halpert, Houston, Texas) Some years ago Dr. Chandler Foot pointed out that in urothelium at times columnar cell islands can be made out. For several years I have been watching for these islands of columnar epithelium within the urothelium, and have seen them quite frequently, so that the association Dr. Mostofi makes of columnar cell carcinoma occurring in urothelium is likely.

(Dr. Otto Saphir, Chicago, Ill.) There are several questions I would like to ask: First, what was the clinical diagnosis in these cases: primary carcinoma of the bladder or of the prostate? Were any metastases found at autopsy in some of these cases? Then I would like to know whether or not Dr. Mostofi knows how often urachal structures are found in the wall of the urinary bladder. In 10 cases which I examined, I found such structures in 8. I would like to know whether the prostate was involved in these cases, and whether there was an elevation of acid phosphatase. Are there any carcinomas of the prostate which are mucinous in type? Do you think that the finding of transitional cell carcinoma and adenocarcinoma, side by side, may be taken as definite evidence of arising in the urinary bladder? Do you think these adenocarcinomas can be explained on the basis of de-differentiation and of arising from the cell nests of Brunn and Limbeck? I have seen several adenocarcinomas arising in the bladder, but I have seen only one instance of diffusely infiltrating mucinous carcinoma with signet-ring shaped tumor cells. The prostate was involved secondarily, and the clinical diagnosis had been carcinoma of the prostate.

(Dr. Mostofi) I do not know that I will be able to answer all of the questions Dr. Saphir has asked. The clinical diagnosis in all but 2 cases was carcinoma of the bladder. In these 2 cases, although the patients had had bladder symptoms, a urologic examination was not done and the diagnosis was made at autopsy. None of them had been clinically diagnosed as carcinoma of the prostate.

In the group of primary adenocarcinoma of bladder there were metastases in 5 and in every case the regional lymph nodes were involved. When the intestinal tract was involved, despite the fact that both the urologist and the contributing pathologist had considered the bladder as the primary site, the case was not included in the study. Furthermore, cases of carcinoma of prostate in which a bladder tumor also was found and on biopsy showed an adenocarcinoma were not included.

I agree with Dr. Saphir's finding of urachal remnants. These are very common if one looks for them.

Four of the primary adenocarcinomas were in the trigone and 2 in the bladder neck where they may have involved the prostate; but I do not have that information available. No phosphatase determinations were done and clinically there was no evidence of a carcinoma of prostate.

In 7 of these 25 patients transitional carcinoma was present in other areas and we found the same thing you reported a number of years ago.

We have seen a rare carcinoma of prostate which is mucus-producing.

Cystitis glandularis and cystica are found in inflammatory conditions of the bladder, in association with carcinoma of the bladder and in portions of bladder overlying secondary tumors. I believe that when these glandular structures (cystitis glandularis) are in the bladder proper they arise from the bladder mucosa.

The transitional epithelium of the bladder is capable of becoming columnar and mucus-producing, and, since it is derived from columnar epithelium of the hindgut, de-differentiation may explain these tumors. According to some anatomists, however, columnar epithelium is a normal component of the vesical epithelium and, if so, one need not postulate de-differentiation.

Fifteen of the 25 primary adenocarcinomas of the bladder were infiltrating and of course all of the urachal adenocarcinomas were.

MAZOPLASIA. J. Gershon-Cohen (by invitation) and Helen Ingleby (by invitation), Philadelphia, Pa.

Abstract. A classic description of mazoplasia was given by Cheatle and Cutler in 1931. Unfortunately, later writers unfamiliar with whole sections of breast misused the term and today it has largely lost its meaning. Each of a series of 150 breast cases which came to operation was studied by radiology and pathology. After examination of paraffin and frozen sections, the entire specimen was cut serially by the slicer method. It soon became obvious—and the conclusion was reached independently by radiologist and pathologist—that a well defined group of cases detached themselves from the general mass usually labelled "chronic mastitis" by surgeons. This group coincided with the mazoplasia of Cheatle.

Essentially, mazoplasia is hyperplasia of duct tissue. The cases fall into groups according to the predominating type of proliferation, but it must be understood that pure types are rare and the groups shade imperceptibly into each other.

Group I. Abnormal proliferation of intraductal (myo-epithelial) tissue so that the ducts become surrounded by a fibrillary zone many times the lumen of duct and its lining epithelium. Lobules are poorly developed or even absent. Microscopic fibroadenomas are common.

Group II. Characterized by formation of small or large cysts. Lobules may be represented by groups of cystic ductules. There is usually more or less myo-epithelial hyperplasia as in group I. The lining of the cysts is smooth.

Group III. Resembles I, but with one or more clinically obvious fibroadenomas.

The x-ray appearance of mazoplastic breasts of group I is characterized by a uniform density of the mammary tissue. Fat deposits may interlace this homogeneous structure, but this appearance is quite different from the fibrous interweaving of the suspensory ligament so clearly seen in the older breast, especially in the presence of atrophy and cysts, as in group II. The bases of the suspensory ligament that traverse the subcutaneous layer are not swollen or thick as in cystic disease or more advanced mazoplasia II. No isolated infiltrations or masses are seen as in fibroadenomas (group III) or cysts (group II).

Mazoplasia is an exaggeration of the state found normally at birth, puberty, and early pregnancy. A similar lesion is found in hyperplasia of the male breast. It is due to hormone imbalance and can be induced by injections of estrogenic substance. It precedes fibroadenoma, but as far as is known is not a direct cause of carcinoma. The changes are brought about by hyperplasia of the undifferentiated cells of the basal layer in the ducts. These cells give rise, as was first shown by Peyron, to both epithelial and myo-epithelial cells. The transition can be followed in many of our sections.

Discussion

(Dr. Otto Saphir, Chicago, Ill.) Can you tell us what the derivation of the term "mazoplasia" is? I think the term was used originally by Cheatle and Cutler.

(Dr. Ingleby) Yes. That is why I used it.

(Dr. Saphir) I could not find out why they called it mazoplasia.

(Dr. Ingleby) We call these lesions mazoplasia in honor of Cheatle, who gave a classical description of the condition, and I think that name should be retained. The derivation I believe is from the Greek: mazo, a breast, and plasia, a growth of tissue.

THE RÔLE OF THE VERTEBRAL VENOUS SYSTEM IN THE SPINAL METASTASES OF CANCER. Dale Rex Coman and (by invitation) Robert P. deLong, Philadelphia, Pa.

Abstract. The frequency with which cancers of the prostate, breast, and thyroid gland metastasize to the bones of the vertebral column, skull, and pelvis cannot be explained satisfactorily on the assumption of transpulmonary passage of tumor cell emboli, with subsequent selective localization in these areas, because of hypothetically favorable "soil" conditions. The suggestion of O. V. Batson that the vertebral veins might offer a pathway directly to these areas, by-passing the lungs, was explored. Tumor cell suspensions were introduced into the femoral veins of rats and rabbits while slight abdominal pressure was applied. Walker rat carcinoma 256 and the V_2 carcinoma of rabbits were used. Tumors appeared in the vertebral columns of the experimental animals, whereas in the controls, to which pressure was not applied, the resulting tumors appeared only in the lungs. Tumor cell emboli were found in the ramifications of the vertebral veins in the experimental animals. From these experiments it is concluded that the frequency of vertebral metastases in cancer depends upon entrance of tumor cell emboli into the vertebral venous system through which they are carried to the vertebrae, skull, and pelvic bones directly, without traversing the lungs.

Discussion

(Question from floor.) In carcinoma of the prostate there is a predilection for skeletal metastases; they are not limited to the spinal column, and I think this occurs also in carcinoma of the lung. I should like to ask if Dr. Coman is able to explain this predilection for the whole skeleton.

(Dr. Anderson Nettleship, Little Rock, Ark.) Have you found any evidence of cerebral metastases in these case studies? It is often contended that the vertebral venous system is primarily the route of metastases of tumors into the brain, when they originate in the abdominal cavity. This does not include cases of secondary implants in the lung. Our explanation has been that there are probably small areas of tumor in the lung, but we miss them at the autopsy. We think this is one of the ways in which metastases reach the cerebral cavity.

(Dr. Coman) As regards the formation of metastases in other bones than the bones of the skull, vertebrae and spine, I do not think we made any suggestions at all. It may be that these other metastases arise secondarily in the lung. There may be other groups; I do not know that.

There were cerebral metastases in some of our animals. There were a few in which we had tumors in the skull and some intracranial tumors that were within the circulation of the brain.

(Dr. Nettleship) How do you think they got there?

(Dr. Coman) I am not certain. They occurred only in those animals in which abdominal pressure was applied and in which cells did get into the vertebral system.

THE MORPHOLOGY OF THE MALIGNANT SQUAMOUS CELL.* James W. Reagan (by invitation) and Richard D. Moore (by invitation), Cleveland, Ohio.

Abstract. This is a detailed morphologic study of 6,000 neoplastic cells arising in

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

squamous cell carcinoma as seen in tissue spreads obtained by scraping the uterine cervix and aspirating the contents of the cervical canal. The size and configuration of the cells and their nuclei are considered, together with detailed observations on the nuclear components including classification of the chromatin patterns encountered. The relative importance and significance of the cytologic changes are discussed and the findings are correlated with those obtained by histopathologic examination.

Discussion

(Commander W. W. Ayres, Bethesda, Md.) We examined a large number of malignant cells stained with azure C, and noted nucleoli in nearly all of these cells. Apparently the process of fixation results in the deposition of chromatin around the nucleoli which results in obscuring the nucleoli. I rather suspect that the chromatin strands shown in your preparations indicate deposition of chromatin about nucleoli.

(Dr. Reagan) It is exceedingly difficult to distinguish the so-called false nucleolus from the true nucleolus because of the presence of nucleolus-associated chromatin. When we apply the Feulgen reaction, however, it is easier. Actually we accepted only structures which were definitely recognizable as nucleoli and excluded all the others.

A Microfluorometric Method under Development for the Scanning and the Detection of Cancer Cells in Vaginal Smears. Robert C. Mellors and (by invitation) George N. Papanicolaou and Adele Glassman, New York, N.Y.

Abstract. The reflecting microscope and associated optical and electronic methods are being used to investigate the physical and chemical properties of the intact cell. In this report attention is given to the physical detection of the cancer cell in material which is of importance in the cytologic diagnosis of cancer. For this a study was undertaken of specially prepared smears of exfoliated cells of the vagina and cervix of 40 patients with and without epidermoid cancer of the cervix. Analytic methods were applied to the measurement of (1) the ultraviolet light absorption in *unstained* cells, and (2) the fluorescence of cells *selectively stained* with a basic fluorochrome. The results follow.

1. The ultraviolet light absorption patterns from 240 to 350 mμ were recorded across the entire cellular diameters. The average absorption, generally maximum at 260 mμ, was much greater in the nuclei of cancer cells and abnormal cells as compared with those of normal or atypical epithelial cells.

2. The intensity of fluorescence was photometrically determined in cells stained with a basic fluorochrome chemically bound to nuclear chromatin by the control of pH (6.3), concentration, time and temperature of the reaction. There was significantly greater fluorescence intensity, averaged over areas with diameters of 5 to 10 μ, in the nuclei of cancer cells and abnormal cells as compared with those of normal or atypical cells.

3. The data in (1) bear a linear relation to those in (2); both are measurements of the relative amounts of chromatin in nuclei.

4. The probability of occurrence by chance alone of the differences between cancer cells and normal epithelial cells with respect to the average absorption (1) or fluorescence (2) is of the order of 1 in 10^4.

5. Subsidiary steps aside from the controlled staining with basic fluorochrome have been investigated and include: (a) the peroxidase reaction to quench the fluorescence of clusters of polymorphonuclear leukocytes, and (b) masking groups such as the sulfates of mucoproteins which are more strongly acidic than the phosphates of nuclear chromatin by causing them to react with a non-fluorescent basic dye at pH 3.2 before staining with basic fluorochrome at pH 6.3.

6. From practical and theoretic considerations it appears that optical and electronic

instrumentation for (2), a microfluorometric method, is feasible for the scanning of cells in smears at the rate of 10^4 to 10^5 cells per minute.

7. Preliminary application of the microfluorometric method has been made to other material: colonic washings, pleural and peritoneal fluid, and urine sediment.

Discussion

(Dr. Hans Popper, Chicago, Ill.) This very ingenious method of Dr. Mellors and Dr. Papanicolaou raises a few questions independent of the scanning method for the quick detection of tumor cells. If I understood it right, the staining was applied primarily to the nucleus. I would like to know whether specific stains for the desoxypentose nucleic acid of the nuclear chromatin were used which would not stain the pentose nucleic acid of the cytoplasm. There are only a few stains which permit such a differentiation, and in our experience with fluorescent dyes we came across hardly any which will stain only the chromatin without cytoplasmic material. I wonder whether measurements of the cytoplasmic material would not be almost a better method for the recognition of tumor cells in view of the characteristic cytoplasmic basophilia of tumor cells and since their PNA is more elevated than their DNA. To me it is especially gratifying to see that the ultraviolet absorption runs parallel to the fluorescence, because I feel this is the most conclusive point. Finally, would it be possible to improve this method and make it even more specific by giving the patients an intravenous injection of a fluorescent dye before the material is obtained? By this procedure, one would get an intravital preparation. There are fluorescent dyes available for intravital staining which are not toxic.

(Dr. Mellors) Any number of basic fluorescent dyes can be used in this technic. The conditions of staining will be such that the dye will bind with any nucleic acid, whether it be DNA or PNA—and that is what occurs. Because of the frequent occurrence of many stripped nuclei—cells that have only nuclei and very little cytoplasm—in smears of this type, and because nuclear abnormality is the most significant single cytologic diagnostic criterion, we have emphasized the measurement of the nuclear material, *i.e.*, chromatin. In actual fact, our method cannot exclude one part of the cell from another. Everything in the cell is scanned, although, as was shown, the majority of the fluorescence is contributed by the nuclear chromatin; but as was shown also, there was a contribution by the cytoplasm. Cells which have a predominant basophilia in the cytoplasm will have fluorescence in the cytoplasm, as well as in the nucleus. The technic in my opinion appears to be simple enough as it is. The materials are collected and fixed in a routine way, and the staining procedure requires only a few minutes. The intravenous administration of dye probably would not lead to sufficient localization of the dye in the particular cells of interest.

THE CYTOLOGY OF INCLUSION BODY PAPILLOMAS YIELDING VIRUS-LIKE PARTICLES BY ELECTRON MICROSCOPY. Henry Bunting and (by invitation) Maurice J. Strauss and Joseph L. Melnick, New Haven, Conn.

Abstract. The cells of certain plantar and common warts have been found to contain eosinophilic intranuclear inclusion bodies and characteristic cytoplasmic masses. These warts when ground and suspended in water have yielded particles visible by the electron microscope in contrast to the warts that do not have these features. The particles are spherical and exhibit a tendency to aggregate in a crystalline pattern. They measure 68 $m\mu$ when single and 52 $m\mu$ when clustered. The inclusion body is single, round, and surrounded by a faint, clear zone; it first appears in the nuclei of cells in the first or second layer above the basal layer and increases in size (from 1.6 to 3.2 μ in diameter) by the time the cell reaches the upper malpighian layers. The inclusion body is Feulgen negative, is unaffected by ribonuclease, and

gives no specific absorption at 2650 Å, but non-specific protein absorption at 2800 Å. Nucleoli can be found in the same cells that possess the nuclear inclusion body. The development and differentiation of cells containing the inclusion body are markedly affected. The cells are apparently incapable of division and become larger than normal. The nucleus also is larger and frequently distorted. The cytoplasm contains many small bodies that increase in size and become fused in the cells of the upper malpighian layer into conspicuous block-like structures. Vacuoles are present within them which behave like myelin figures, suggesting the presence of a lipid, although none has been demonstrated by sudan black. Intercellular bridges do not form between two affected cells. Keratohyaline granules do not develop and the nuclei persist as pyknotic structures in the stratum corneum (parakeratosis). The changes described may be present in the entire wart or at times in only three or four fronds, the others exhibiting only acanthosis and hyperkeratosis without the nuclear and cytoplasmic features.

Discussion

(Dr. Herbert Z. Lund, Cleveland, Ohio) I would like to ask if these peculiar cytoplasmic bodies have been seen anywhere but in heavily keratinized skin, the skin of the feet or the palms? Have they ever been seen in digitate warts? Secondly, if this type of wart is transplanted into a different type of skin would you expect to see these exact changes reduplicated? I have had the feeling that these intracytoplasmic accumulations are peculiar to the site of the wart in heavily keratinized skin.

(Dr. Raymond G. Gottschalk, Houston, Texas) What is the proportion of warts that show these characteristics?

(Dr. Bunting) We have seen these cytoplasmic masses, whether the warts were from the sole of the foot, the palmar surface of the hands, or the backs of the fingers, so I have been unable to correlate the degree of keratinization of the overlying skin with their presence. I have no warts in our collection that have come from anywhere else in the body. The back of the finger is not heavily keratinized, don't you agree?

(Dr. Lund) I think it is more so than, say, the face.

(Dr. Bunting) I will transplant one there the next time instead of to my finger. We have not observed cytoplasmic masses in digitate or filiform warts.

Concerning the percentage of warts that show these characteristics: We found such changes were very frequent in warts from the sole of the foot, occurring in about 44 per cent of the specimens; the percentage is much lower in warts from the hands, something in the order of 4 per cent; this gives an over-all precentage of 13 in a series of 156 consecutive cases. These results will be published shortly.

PATHOLOGIC CHANGES INDUCED EXPERIMENTALLY IN LABORATORY ANIMALS BY MICROORGANISMS RECOVERED FROM THE BLOOD AND TUMOR TISSUES OF HUMAN CASES OF MALIGNANT DISEASE. Lawrence W. Smith and (by invitation) Virginia Wuerthele-Caspe and Eleanor Alexander-Jackson, New York, N.Y., and Newark, N.J.

Abstract. In a study initiated by one of the authors (V.W-C.) in 1947, an organism was recovered from a case of breast cancer presenting many features in common with a similar organism found in a series of scleroderma cases. Tentatively, this was classified as probably a mycobacterium because of its acid-fast characteristic and pleomorphic appearance under various cultural conditions. Up to the present time, this organism has been obtained uniformly from all cases of malignant disease, including the standard experimental animal tumors. To date it has not been recovered from the blood of any normal control case (donors for blood bank). Morphologically similar organisms have been recovered from 2 cases of cystic disease of the breast, but the cultural characteristics and biologic activity have been strikingly different.

Inoculation of experimental animals with small amounts of filtrates from a special broth medium, intraperitoneally or subcutaneously, result in varying pathologic pictures. The lesions may be restricted to the site of inoculation or may show widespread metastases. These differences may be dependent in part on the size of the dose and individual differences in susceptibility or resistance. With intraperitoneal inoculation, one of two types of change is observed—either a widespread diffuse autolysis of all organs without demonstrable focal lesions or a widespread systemic chronic granulomatous process with innumerable foci in the lungs, liver, spleen, and other organs. The characteristic lesion is a pseudocaseous chronic granuloma, usually yellowish gray, quite firm, and showing little or no liquefaction necrosis even centrally. The lesions frequently coalesce or extend by continuity or contiguity to regional structures. Grossly it is impossible to tell whether these are inflammatory or neoplastic. Repeated transfer to successive animals results very frequently in the development of large, progressive, focal lesions with hematogenous metastatic distribution. Grossly, the kidney is relatively uninvolved in most cases, the major lesions being found in the liver and lungs, although no organs are immune. Histologically, these lesions are for the most part of a chronic proliferative nature with a pattern more consistently suggestive of a chronic granulomatous infection than of a true neoplasm. However, occasional cases present morphologic evidence of a transition from this chronic granulomatous proliferative lesion to one of actual neoplasia.

Discussion

(Dr. Helen Ingleby, Philadelphia, Pa.) I would like to ask whether the tumors produced were of the same type in all animals.

(Dr. Anderson Nettleship, Little Rock, Ark.) I realize this is a very old concept and one which has been worked on extensively. It seems as though the die is very clearly cast here; either we are going to have to go back to the early theories and accept that many, if not all, neoplasms are related to bacteria or virus-like infections, or they are not. I can hardly avoid recalling an early paper by Welch in which he remarked that he had spent the evening examining a large group of tumors said to have been caused by fungi. The demonstration of fungi was clear enough, but whether they caused the tumors or not remained a question in his mind. It is going to take far more than was offered here to establish a causative effect. I should like to ask how the authors explain neoplasms of the teratologic group and the tumors which are induced by various carcinogens.

(Dr. J. K. Frenkel, Hamilton, Mont.) May the various lesions shown here co-exist in one animal, or are they taken from various animals? Have you found specific antibodies in the sera of patients to the organisms isolated from them?

(Dr. Smith) In answer to Dr. Ingleby's question, of course we could not cover the whole field in the length of time permitted, and one of the criticisms I knew was going to arise was whether we could reproduce the tumor of origin. I tried to emphasize briefly that we did not necessarily do so. I think it goes right back to Ewing's idea of the potentiality of *any* cell to undergo malignant change. Obviously in the experience of all of us the lymphoid and less differentiated connective tissue cells probably are the more readily stimulated to neoplasia. We have had three instances in which the induced experimental tumor was found to be a carcinoma. They have not been identical with the carcinoma of origin. Most of the malignant tumors have been of the lymphoid series.

Replying to Dr. Nettleship, we obtained about 3 to 4 per cent of rather frankly malignant tumors following 7 to 10 transfers in these animals. We do not believe that the infectious agent represents but one phase of neoplasia. At the moment I would like to express my personal belief that we *cannot* have cancer without an infectious agent, and that the organism we have described is the responsible one; on the other hand, I am equally convinced that we *can* have the organism *without*

cancer, just as we can have immune carriers with any other low-grade infection. We firmly believe that host resistance or susceptibility are the other factors determining the kind of tumor, the site of tumor development, and the degree of malignancy which exists. It may well be that carcinogens, acting on the individual cell, aid in preparing the soil for microbial invasion. I cannot explain teratologic tumors except on the basis of intra-uterine passage of the organism. This latter phenomenon we know occurs in the offspring of inoculated pregnant mice. These studies do nothing to explain that part of the problem.

In reply to Dr. Frenkel's query regarding the occurrence of multiple tumors of different types in the same animal, we can only say that all of these tumors have been individual tumors thus far. We have not had two different kinds in the same animal. In respect to immunologic phenomena, we have been able to demonstrate the formation of anti-serum with this organism, but thus far without a high titer.

DEMONSTRATION BY THE NADI REACTION OF CYTOCHROME OXIDASE ACTIVITY IN CELLS OF THE LYMPHOID AND MYELOID SERIES, OBTAINED FROM NORMAL INDIVIDUALS AND PATIENTS WITH HODGKIN'S DISEASE. George Hoffman (by invitation) and Antonio Rottino, New York, N.Y.

Abstract. By previous studies on the cytochrome oxidase system in lymphoid tissue employing manometric methods, the oxidase activity of the tissue as a whole has been measured. Since such material is not homogeneous but is made up of a number of different cell elements, it seemed advisable to us to study the cytochrome system in each of these elements separately. An old histochemical procedure, the Nadi reaction, results in the precipitation of insoluble indophenol blue at the site of cytochrome oxidase activity. We utilized this reaction for making a differential study of the cytochrome system in the various cells of normal and abnormal lymphoid and myeloid tissue.

Lymphoid cells were obtained from the cut surface of lymph nodes and spleen by squeezing, and myeloid cells were obtained from the buffy coat of blood and bone marrow aspirations. The cellular fluids were mixed with equal parts of a saturated solution of alpha naphthol and 0.2 per cent dimethyl-p-phenylene diamine, the whole being buffered to pH 7.4. The preparations were examined by phase contrast to check the identity of the various cells and by bright field light to check the color of the granules of indophenol blue formed by the reaction.

Within 10 minutes of the time of mixing cells and Nadi reagent, round, bright-blue granules, varying from 0.5 to 2.0 μ in diameter, could be seen in the cytoplasm of all cells. The number of granules varied according to the cell type. In decreasing order of oxidase activity, based roughly on the average number of granules observed, the cells could be listed as follows: monocytes and free reticulum cells, macrophages, myelocytes, lymphocytes, and polymorphonuclear leukocytes. Cells of myelogenous and lymphatic leukemia showed the same activity as their normal counterparts. Sternberg-Reed cells from lymph nodes showing Hodgkin's disease were quite variable in their reaction. The blue granules observed in cells of lymphoid and myeloid tissue corresponded in size, number, and location to cytoplasmic bodies which could be seen by phase contrast in unstained cells and which could be stained supravitally with Janus green B. It has been demonstrated by manometric experiments on ultracentrifuge fractions of disrupted cells, that the highest concentration of cytochrome oxidase activity is in the large granule (mitochondrial) fraction. The staining reaction (*i.e.*, the number of blue granules) in the cells of lymphoid and myeloid tissues is much less intense than that noted in cells of myocardium, liver, and kidney. These latter are literally loaded with granules after contact with the Nadi reagents. Quantitative manometric experiments have shown that such tissues have some ten times the activity of lymph nodes and spleen. The low concentration of cytochrome oxidase in cells of the lymphoid and myeloid systems may be interpreted to mean

that either their energy requirements are small as compared with those of heart, liver, and kidney, or that sources of energy other than that derived from the oxidation of carbohydrates are available to these cells, *e.g.*, energy-rich phosphate bonds in the form of adenosine triphosphate. No experimental evidence seems to be available concerning this question.

Discussion

(Dr. Robert C. Mellors, New York, N.Y.) I would like to ask if cyanide or other inhibitors were used to enhance the specificity of this reaction for cytochrome oxidase.

(Dr. Hoffman) For purposes of brevity I did not go into the technical details. Both cyanide and azide were used.

INVESTIGATION FOR THE PRESENCE OF FUNGI IN HUMAN NEOPLASTIC TISSUES. H. Mescon, J. W. Eiman, and A. M. Kligman (all by invitation), Philadelphia, Pa.

Abstract. In the course of performing several histochemical tests on biopsies of various skin lesions it was found that the Hotchkiss-McManus stain caused fungi to take up the dye and that very few tissue elements stained. With the Hotchkiss-McManus technic certain complex polysaccharides appear red. The chitin-cellulose wall of fungi is such a substance, therefore it stains. Tissue of deep and superficial human and animal mycoses were examined with this method and the organisms stain differentially. Almost all inflammatory cells are uncolored. This procedure was used to demonstrate forms and numbers of fungi in tissues that were heretofore not known to exist.

Diller and Fisher reported that "fungi imperfecti related to the Blastomycete group have been isolated from all types of tumors including human neoplasms and from mouse leukemias. All types of human neoplasms thus far tested produced fungus growths. Furthermore, spore forms are microscopically demonstrable in many types of human tumors that have not been cultured." In view of this, 63 human visceral tumors removed by surgery were cultured for fungi and the Hotchkiss-McManus stain was applied to tissue sections of these and to an additional 140 sections of tumors. The latter group included 10 basal cell carcinomas, 10 squamous cell carcinomas, 10 cases of leukemia, 10 of Hodgkin's disease, and 28 of sarcoid. Of the cultures, approximately 35 per cent showed no growth and the remainder revealed an assortment of organisms ordinarily regarded as contaminants. Sixteen different species of fungi were cultured from the 50 malignant tumors. No one species was cultured in more than 6 per cent of the tumors. Benign growths yielded similar results. Microscopically, no fungi could be determined in the tumor tissue.

Discussion

(Dr. J. F. A. McManus, Charlottesville, Va.) I wonder if, beside a culture of the tumors, there was a culture taken of apparently normal tissue to see whether something like the same organisms might be present.

(Dr. Otto Saphir, Chicago, Ill.) This is a very interesting study, particularly in the light of the one we heard a few minutes ago. I think we should be very careful in our judgment, because we really do not know whether there is an agent involved in the origin of the tumors. What I want to point out particularly is that in the first paper the fungi could not be identified, while in the second presentation all the fungi are definitely identified. I would like to know whether some of the fungi shown here are pathogenic, and whether any studies were made in regard to the immunologic aspect.

(Dr. Mescon) In reply to Dr. McManus's question, we did not do control studies on normal tissue. This is a deficiency on our part. However, in the same laboratory where these studies were undertaken, the mycology laboratory, the fungi which we

did isolate from tumors are those organisms which are encountered in our routine mycologic work and are commonly regarded as contaminants.

Dr. Saphir, we have not done any immunologic studies on these tumors. In so far as their pathogenicity is concerned, most of these fungi in the past have been reported in isolated cases as being the cause of a fungous disease, but all of them have been recovered, as well, from completely normal tissue and from tissue in which they obviously play no part, as far as we know. It must be emphasized that our study is limited only to fungi.

LIMITATIONS OF THE ANTERIOR CHAMBER AS A TUMOR TRANSPLANTATION SITE, USING A SERIAL SACRIFICE METHOD. E. J. Eichwald (by invitation) and H. Y. Chang (by invitation), Salt Lake City, Utah.

Abstract. Several investigators have failed to verify Greene's dictum that heterotransplantability is a basic characteristic of cancerous tissue, as indicated by successful transplantation to the anterior chamber of the guinea-pig. "Takes" were achieved only sporadically, or not at all. Recent studies indicate that successful transplantation of cancers to the guinea-pig eye is more common than is believed by many workers, although it does not occur with all cancers. This became apparent after abandoning the conventional method of conducting these experiments. This method consisted of a gross evaluation of the eye at regular intervals, and its removal for microscopic verification when tumor growth was suspected. When this method was replaced by one of serial sacrifice of the hosts regardless of the gross appearance of the eye, unsuspected tumor growth often was found.

Seventy-five guinea-pigs with anterior chamber transplants of mouse sarcoma 37 were sacrificed at daily intervals in groups of 5. Growing tumor was found in all animals sacrificed between the sixth and ninth day, suggesting that the tumor grew, or would have grown, in all animals during this period. Using the conventional method, growth of this tumor was detected in only 44 per cent of over 900 animals. Study of the events early after transplantation revealed why many growing tumors escape detection. The grafts rapidly became necrotic except for a few viable peripheral cells. Some of these penetrated the iris, and others settled in the crevices of the pectinate ligament. In both locations they proliferated readily. It appears that tumors in the pectinate ligament may regress before they protrude into the visible portion of the anterior chamber; a tumor in the iris may never reach the size of the necrotic graft in front of it. Exudate, a corneal pannus, or ulceration may mask a growing tumor.

To determine whether the method of serial sacrifice would permit detection of successful transplantation of other and perhaps of all cancers, and to other foreign hosts, 3 mouse tumors and 4 human cancers were transplanted to 25 guinea-pigs each, the hosts being serially sacrificed. One of the mouse tumors, neuroblastoma C 1300, heretofore believed to be not transplantable to the guinea-pig eye, appeared to grow in all inoculated animals. Transplantation of the other mouse and the human tumors was not successful. Mouse sarcoma 37 was transplanted successfully to the eyes of rats, hamsters, and rabbits; and to cats and dogs without success.

It is concluded that (1) the method of serial sacrifice of hosts permits detection of a higher number of successful transplantations than the old method of observation; (2) the evidence suggests that either all grafts of a tumor grow, or none; (3) heterotransplantability is not a basic characteristic of cancerous tissue.

Discussion

(Dr. Abraham Towbin, Columbus, Ohio) In our laboratory we had similar difficulties in evaluating results. Although this pattern of growth applies to sarcoma 37, I do not think it is uniform in all tumors; actual growth from the margin into the aqueous humor does occur. You have not told us clearly what your criteria of a suc-

cessful transplantation are. Surely you do not mean just a few proliferating cells. It must be a tumor growing in an organized way with stroma contributed by the host.

(Dr. George Milles, Chicago, Ill.) How do you differentiate transplanted tumor cells from cells contributed by the host?

(Dr. Eichwald) We can only state that in the tumors studied we saw this pattern. I do not know whether in other tumors it occurs in a different pattern.

The second question: whether the cells we see are tumor cells or host cells has plagued us considerably. However, the method of serial sacrifice permits a reliable tracing from cells of the original graft to those rapidly proliferating after vascularization by host vessels.

LYMPHANGIO-ENDOTHELIAL STRUCTURE OF CERTAIN THYMIC TUMORS. David S. Hubbell (by invitation), Averill A. Liebow, and Gustaf E. Lindskog (by invitation), New Haven, Conn.

Abstract. Three tumors from the region of the thymus have been encountered whose structure suggests a large contribution of endothelium of lymphatics. Two of these neoplasms occurred in aged individuals and a third in a 17-year-old Negro girl. None were associated with myasthenia. The cells interpreted as lymphangio-endothelium occurred (1) as interlacing spindle-shaped elements; (2) as similar cells, but arranged in part about minute lumina; (3) as larger elements in honeycomb arrangement in the walls of large spaces, or as a single layer of flattened cells lining apparent lymphatic channels. Hassall's corpuscles were found in only one tumor, although otherwise the fundamental structure was the same in all. Review of illustrated published articles suggests that the larger cells of these tumors generally have been considered to be of epithelial origin, but the presence of the various transitional forms in the present series suggests their lymphangio-endothelial origin.

Discussion

(Dr. Joseph A. Kasper, Grosse Pointe, Mich.) I would like to ask if any metastases were associated with these tumors.

(Dr. Jesse L. Carr, San Francisco, Calif.) This is a very interesting study of these tumors. We have in approximately 22 years collected only two of a similar pattern. In your endothelial studies I would like to ask if you can corroborate or disprove the statement that there are no afferent lymphatics in the thymic nodules.

(Dr. Liebow) In regard to metastases, one of these patients, the 71-year-old person, died very soon after the operation of unrelated causes. In the other two, 2 years have elapsed since operation in one case, and 7 months in the other. We do not have any long-range follow-up. Similar tumors which have been described by Seybold have shown very little tendency to metastasis, certainly not beyond local implantation upon the pleura, as occurred in 2 of their 45 instances. There has been no evidence of metastasis by the blood stream.

In regard to Dr. Carr's comments as to the presence of lymphatics in the thymus, they most certainly do exist in the stroma of the gland as shown by the presence of apparently empty channels lined by endothelium and possessing a muscular wall. Moreover, we are not dealing with normal thymic material. Although it may well be that the medullary substance of the normal thymus does not contain lymphatics, just as the spleen contains few lymphatics, nevertheless the stroma may be involved in the neoplastic process. The histologic pattern would suggest that lymphangio-endothelium makes a contribution to these lesions.

GASTRIC CARCINOMA: A MULTICENTRIC LESION. William T. Collins (by invitation) and Edward A. Gall, Cincinnati, Ohio.

Abstract. This study is based upon a histologic analysis of large (8 by 12 cm.) tissue sections of 117 specimens of gastric carcinoma. Most sections included the

entire breadth of the tumor and a variable amount of grossly uninvolved mucosa at either margin. Primary attention was directed at the occurrence of independent foci of pre-invasive carcinoma at a distance from the margin of the tumor; non-invasive neoplastic alterations within the mucosa contiguous to the cancer; and segmental pleomorphism of the histologic pattern within the major tumor itself. Fifty-nine of the 117 specimens (50 per cent) revealed phenomena of this nature alone or in combination. These observations lend some credence to the hypothesis that carcinoma of the stomach results in many instances from the coalescence of multiple independent foci of origin.

Discussion

(Dr. Anderson Nettleship, Little Rock, Ark.) This question of the multicentricity of lesions is one of such importance, and one which has been so sadly neglected in this country, that I think it might be emphasized. In 1946 we studied a large series of early cancers. The idea was to study them as early as possible in order to learn about their point or points of origin. We studied early carcinomas of the cervix, skin, and breast. All very clearly showed multicentric origin. Of course, we always face the problem of lymphatic spread, and some pathologists find this such a hazard that they refuse to accept the concept of multicentricity. It seems to me with almost any carcinogen there is the possibility of many areas simultaneously changing into a pattern which will give rise to a neoplasm. It does not mean that of necessity neoplasms will originate in the altered tissue background. The proper milieu may be present and still only a single or few foci become carcinomatous. But multicentricity seemingly plays a part in the origin of certain tumors. I think the present studies are very fine, and I would like to compliment the authors.

(Dr. Emmerich von Haam, Columbus, Ohio) Some time ago Professor Sternberg was interested in the same subject, and in order to obtain large sections he rolled thin slices of stomach up in bandage-like fashion and prepared sections with the microtome. He observed pictures similar to those demonstrated by Dr. Collins, but Dr. Sternberg was too conservative in his interpretation and could not eliminate the possibility of implantation metastasis.

(Dr. Collins) This is not a new subject; over 40 years ago it provoked a great controversy in the German literature. Several German investigators have demonstrated the occurrence of independent neoplastic foci of microscopic sizes in gastric cancer. The theory of multicentric origin of cancer is not accepted by all, although evidence is accumulating that cancer in various organs develops in this fashion.

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COARCTATION OF THE AORTA AS A CAUSE OF DEATH IN EARLY INFANCY. Robert C. Bahn (by invitation), James W. DuShane (by invitation), and Jesse E. Edwards, Rochester, Minn.

Abstract. Three young infant patients died of left ventricular cardiac failure as the result of coarctation of the aorta with poorly developed collateral circulation. In each instance the site of coarctation lay opposite a closed or closing ductus arteriosus. The position of the coarctation with respect to the location of the entrance of the ductus arteriosus into the aorta will determine whether collateral vessels adequate to by-pass the coarctation will develop in fetal life. If the coarctation lies beyond the ductus arteriosus, collateral vessels necessarily develop during fetal life if the fetus survives. In cases in which the coarctation lies proximal to the entrance of the ductus arteriosus the fetal circulation is little, if at all, disturbed, and collateral vessels do not develop. In these patients, after the ductus arteriosus closes, the presence of coarctation with inadequately developed collateral vessels causes left ventricular

strain and failure. In cases in which the coarctation lies opposite the ductus arteriosus, adequate collateral vessels develop in some but not in others. Whether collateral vessels develop in cases of this type seems to depend on the direction of predominant flow from the ductus into the aorta. When the predominant flow is into the descending aorta, collateral vessels will not develop. In those cases in which the ductus flow is predominantly into the proximal aorta, collateral vessels will develop. The 3 patients concerned in this study constituted examples of coarctation of the aorta opposite the ductus in which inadequate collateral circulation had formed. The effects of this in the presence of a closed or closing ductus arteriosus were obstructive hypertension, left ventricular strain, and, ultimately, left ventricular failure.

HISTOCHEMICAL DEMONSTRATION OF DEHYDROGENASE ACTIVITY IN HUMAN MAMMARY CANCER. Maurice M. Black and (by invitation) Francis D. Speer, New York, N.Y.

Abstract. In a study of the dehydrogenase activity of slices of mouse mammary cancer as evidenced by the reduction of triphenyltetrazolium chloride (TTC), it was found that such activity was limited almost exclusively to the stromal capillaries and fibroblasts. Such activity was not inhibited by fluoride, malonate, or azide. No such findings were encountered in a wide variety of normal tissues.

In the present study similar methods were applied to an investigation of the dehydrogenase activity of human breast carcinoma as well as homologous normal breast tissue. In addition, such studies were applied to lymphosarcoma, normal lymphoid tissues, and granulomata. Unlike the findings in mouse mammary carcinoma, the human breast tumor cells showed intracellular TTC reduction in many instances. It was found that in different cases or in different areas of the same tumor the intracellular formazan might be: (a) absent, (b) in the form of fine granules, (c) imparting a red color to preformed lipid, (d) irregular or elongated crystals. The stromal activity appeared to be inversely related to the dehydrogenase activity of the tumor cells in the area. Neither the stromal nor tumor cell reduction of TTC was appreciably inhibited by fluoride, malonate, or azide in the concentrations employed. Control breast parenchyma or benign adenomas showed only minimal endogenous staining of the stroma and essentially none in the presence of the aforementioned inhibitors.

Normal lymphoid tissue or granulomatous masses showed only slight dehydrogenase activity and this activity was inhibited by the enzyme inhibitors mentioned. In contrast, lymphosarcomatous tissue showed an increased dehydrogenase activity, not appreciably inhibited by the inhibitors. Frozen section examination of the latter tissue indicated a definite participation of the stromal elements in the total dehydrogenase activity.

These findings indicate the need to correlate quantitative measurements of respiratory enzyme activity of neoplastic tissues with histochemical evaluation of the participating elements. Further, they demonstrate a unique tumor-stromal relationship in human as well as in mouse cancer. At present no conclusions are possible in regard to the significance of this phenomenon and the biologic properties of malignant tumors.

LACK OF CORRELATION BETWEEN TUBERCULIN SENSITIVITY AND SPECIFIC ANTIBODY FORMATION INDUCED BY BCG IN SARCOIDOSIS. W. H. Carnes and (by invitation) J. B. McKewen, A. M. Fisher, and S. Raffel, Baltimore, Md.

Abstract. It has been reported previously that among patients with sarcoidosis there are a low incidence and low titers of complement-fixing antibodies to tuberculosis. It was concluded that this difference constituted additional evidence against the theory of the tuberculous etiology of sarcoidosis, unless these patients had an impaired ability to produce or maintain circulating antibody. The latter possibility

could not be dismissed because there is evidence of defective response to antigenic stimulus in sarcoidosis. Not only is relative anergy to tuberculin an established characteristic of the disease, but also there are several reports of the persistence of anergy after vaccination with avirulent bacilli which ordinarily induces sensitivity in normal individuals. Hence the titers of complement-fixing antibody to tuberculoprotein and the response to intradermal tuberculin have been investigated in a group of 11 cases of sarcoidosis after vaccination with BCG. All patients gave negative tests to 1 mg. O. T. prior to vaccination and no positive results were elicited with doses of 0.1 to 10 mg. O. T. at various intervals from 1 to 14 months following vaccination. In contrast, each of 19 healthy control subjects, similarly vaccinated, developed a positive tuberculin test within this span of time. Complement-fixations with culture medium protein of $H_{37}Rv$ were negative in both groups prior to vaccination, low titers of antibody were demonstrated in 6 of the 11 sarcoid patients and in 7 of 19 controls. There was no significant difference between the groups in this respect. It is concluded that the low incidence and titers of antibody to tuberculoprotein formerly found (and currently confirmed) in sarcoidosis are a valid evidence against its tuberculous etiology.

COMPARATIVE EFFECTS OF 400 KV. AND 24 MEV. (BETATRON) X-RAYS ON THE EPIPHYSEAL CARTILAGE OF YOUNG RATS. Earl W. Cauldwell (by invitation) and John S. Laughlin (by invitation), Chicago, Ill.

Abstract. In view of the fact that x-rays of different energies produce varying quantitative biologic effects at equivalent doses, the bio-assay method was used to study the results of high energy x-rays of betatron origin. This required a comparison of local and total body effects on rats exposed to homogeneous doses of radiation from a conventional source and from the betatron. The dose-response of the 400 kv. treated animals was used as the standard of comparison. This report will be concerned only with femoral and tibial growth and with the histologic changes in the epiphyseal cartilages at the knee joint, which area had been exposed to local irradiation.

Over 500 albino rats, 28 to 30 days old and of 66 gm. average weight, were subjected in parallel series to doses of x-rays ranging from 200 r. to 5000 r. at 400 kv. and 24 mev. Animals were sacrificed for histologic study at regular intervals up to 150 days. Growth changes were followed by weekly roentgenograms. The growth data were based on direct and roentgenogram measurements. When the ratios of differences in lengths of non-irradiated left and irradiated right long bones were plotted against the logarithm of time, three well defined groupings were revealed: (1) Those showing a minimal effect, with adequate recovery and growth, represented by the 200 r. to 600 r. (400 kv.), and 200 r. to 1000 r. (24 mev.) series; (2) an intermediate effect, with less adequate recovery, in the 800 r. to 1500 r. (400 kv.), and 1500 r. to 3000 r. (24 mev.) groups; and (3) a maximal effect, with negligible post-irradiation growth, in the 2000 r. to 5000 r. (400 kv.), and 3300 r. and 5000 r. (24 mev.) groups. When the slopes of the above plots were separately plotted against doses in roentgens, the curves differed chiefly in the units of dose. The ratio of units represented a parameter which permitted conversion of betatron dose into conventional dose with respect to the biologic test reaction studied. The equivalent factor had the numerical value of ≈ 0.6.

A histologic evaluation of changes in epiphyseal cartilage based on degenerative and regenerative response in cartilage cells, alterations in matrix, disturbances of osteoblast and osteoclast activities, and changes in metaphyseal architecture, revealed a definite quantitative difference at equivalent dosages in the two major groups, and correlated rather closely with the observed growth changes. In spite of

comparable stunting effects in the maximum response groups, cartilage changes in the 5000 r., 24 mev. animals more closely resembled those in the 3000 r., 400 kv. groups, and only the betatron series showed significant nodular reactivation in the epiphyseal cartilage.

NEUROPATHOLOGIC LESIONS OF ERYTHROBLASTOSIS FETALIS IN RELATION TO NUCLEAR DEAFNESS. William B. Dublin, Fort Logan, Colo.

Abstract. In erythroblastosis fetalis, the principal manifestations include hemolytic anemia, hydrops, and encephalopathy. The last is widespread, involving cerebral cortical areas as well as basal ganglia, brain stem, and cerebellum. The mechanism of injury is anoxic, through intravascular hemolysis. The distribution and gradient of severity of lesions is the same as that in other essentially anoxic lesions, such as carbon monoxide asphyxia. Those centers injured relatively severely may show golden pigmentation, or *Kernicterus*. In children surviving erythroblastosis fetalis, this widespread encephalopathy is reflected in various residual symptoms; among these are mental deficiency, spastic diplegia, and choreo-athetosis. Recently it has been found that bilateral perceptive deafness may occur, either in association with one or more of the foregoing manifestations, or as the sole or outstanding residual. Therefore it seemed worth while to carry out a pathologic review of the auditory pathway in kernicteric brains of erythroblastotic infants. The cochlea is under study elsewhere, and report is reserved. Otherwise, injury was found, in distribution and degree of severity, as follows: of the dorsal and ventral cochlear nuclei, severe; of the superior olivary nuclei, moderate; of the nuclei of the inferior colliculi, mild; of the medial geniculate bodies, moderate to severe; of the auditory cortex, mild to moderate. These lesions form a basis for the clinical auditory defect and for its tendency toward bilateral symmetry.

INTRACYTOPLASMIC INCLUSIONS IN THE PANCREAS DUE TO BORIC ACID POISONING. Russell S. Fisher, Baltimore, Md.

Abstract. Boric acid, although a common household remedy, is capable of producing an exfoliative dermatitis and progressive debilitation which leads to death from cachexia or infection. There have been observed 6 infant deaths due to this sequence of events in Baltimore, Md., in the past 5 years. In each case boric acid was added to dusting powder used on simple dermatitis of the diaper-covered area and was apparently absorbed through the irritated skin in sufficient amounts to cause fatal poisoning. The pathologic findings in 2 of these 6 and in 2 cases from other cities are presented. There is described a hitherto unreported lesion in the pancreas in boric acid poisoning. It consists of intracytoplasmic inclusions varying up to 10μ in diameter in the acinar cells. They are rounded, usually homogeneous, eosinophilic masses; some show deeply basophilic granules enclosed in the eosinophilic material. They are believed to represent a degenerative process. Non-specific findings included bronchopneumonia, disseminated phlebothrombosis, adrenal hemorrhage, and cloudy swelling of parenchymatous organs. Attempts to produce the pancreatic lesions in experimental animals are in progress.

A COMPARISON OF HORSE SERUM NEPHRITIS IN RABBITS AND ANTI-KIDNEY SERUM NEPHRITIS IN RABBITS AND RATS, AND THEIR EFFECT UPON THE ADRENAL GLANDS. Carolyn W. Forman (by invitation), William E. Ehrich, and Joseph Seifter (by invitation), New York, N.Y., and Philadelphia, Pa.

Abstract. Nephritis was produced in rats by 1 or 2 injections of 0.5 to 1 cc. of anti-kidney serum produced in rabbits, and in rabbits by 1 or 2 injections of 10 to 20 cc. of horse serum per kg. of body weight or by 1 or repeated injections of a total

of 3.5 to 11 cc. of anti-kidney serum produced in ducks. It was found that the rat and rabbit nephritides differed both functionally and morphologically, and also in their effect upon the adrenal glands. In rats, anti-kidney serum caused the nephrotic syndrome characterized clinically by heavy proteinuria, edema, and hypercholesteremia, and morphologically by damage of the renal epithelium and thickening of the basement membrane; whereas in rabbits, both anti-kidney serum and horse serum caused glomerular nephritis characterized by proliferation of glomerular mesenchyma with or without the deposition in the capsular space of fibrin and subsequent substitution by epithelium, but without destruction of renal epithelium and without significant alteration of the basement membrane. The rat disease was associated with a marked increase in the weight of the adrenal glands; the rabbit nephritis was not. The functional and morphologic differences of the nephritides are demonstrated, and their bearing on human nephritis and nephrosis discussed.

PATHOLOGY OF CHRONIC TOXOPLASMOSIS IN THE GOLDEN HAMSTER. J. K. Frenkel (by invitation), Hamilton, Mont.

Abstract. In most laboratory animals, the majority of the strains of Toxoplasma tested gave rise to acute, fatal infections. Death could be prevented by adequate sulfadiazine therapy. Such survivors could usually be shown to remain infected for many months, even though no clinical signs or symptoms were manifest. However, when using the RH strain of Toxoplasma in hamsters, the majority of animals developed signs of encephalitis. This appeared 1 to 6 months after subcutaneous infection, progressing more or less rapidly to death within a period of weeks or months. The RH strain did not produce such signs of chronic infection in white mice, voles, wood rats, chickens, dogs, and cats. Likewise, 6 other strains of Toxoplasma failed to give rise to progressive disease in either hamsters or certain of the other animals tested. The first clinical signs in hamsters consisted of irritability and hyperactivity, associated with choreiform head movements. Frequently, the head was tilted to one side and the animal would run in circles. When picked up or when eating, involuntary head movements stopped. These signs would persist for various periods, after which they were followed by weakness of the hindlegs, loss of bowel and bladder control, posterior paraplegia, and death. Retinitis, hemorrhage into the anterior chamber, and cataracts developed commonly.

Microscopically, the lesions consisted of microglial nodules, occasionally progressing to necrosis and astrocytic gliosis. Even though the lesions were scattered at random throughout the brain and spinal cord, certain areas such as Ammon's horn were more frequently involved than the cortex. Toxoplasma, although common throughout the brain and often within neuronal cells, were rarely associated with the lesions. The latter are considered to be formed in reaction to ruptured toxoplasma cysts with the individual microorganisms phagocytized and removed. Retinitis and iritis were present with mononuclear exudate, synechiae, and retino-lenticular adhesions leading to destruction of retina, iris, and lens. The choroid showed little inflammatory involvement. Proliferative toxoplasma and cysts were associated with the uveal lesions, and cysts were also seen away from them. Hamsters, succumbing with chronic toxoplasmosis, often showed adrenal necrosis, originating in the medulla and extending into the cortex. In the adrenal gland, proliferative toxoplasma but no cysts were observed. Whereas, organisms in small numbers could be detected by subinoculation of other organs, no specific lesions were observed in them. High titers of toxoplasma antibody (as measured by the dye and complement-fixation tests) were present throughout the chronic stage of the infection. Tests to detect the presence of a contaminating virus were negative. There were no middle ear lesions or other evidence of pleuropneumonia infection.

This chronic progressive disease of the hamster shows some analogy to toxoplas-

mosis of the human infant, and to chronic recurrent chorioretinitis in patients with serologic and immunologic evidence of toxoplasmosis. In all instances progressive lesions are present in the neuro-ectodermal derivatives, whereas the extraneural viscera are essentially free of them, even though they contain small numbers of organisms; circulating antibodies are present and the sulfonamides are without significant effect. Hypersensitivity appears to play a significant rôle in the pathogenesis of these lesions. This infection in the hamster might, therefore, prove a useful model for the study of certain aspects of the human infection.

ENDOCRINE INFLUENCES ON PROTEINURIA IN THE RAT: EFFECT OF HYPOPHYSECTOMY. H. Goodman (by invitation), J. Marmorston, and (by invitation) A. Sellers and S. Smith, III, Los Angeles, Calif.

Abstract. Endocrine effects on the renal excretion of protein have been stressed only recently. Addis reported the production of an intense transient proteinuria by the intraperitoneal injection of the kidney extract, renin, in the normal rat, and the abolition of this proteinuria response to renin after bilateral adrenalectomy. In addition, we have shown that adrenalectomy diminishes the spontaneous proteinuria exhibited by the normal male rat as well as the proteinuria which follows the intraperitoneal injection of serum albumin. The present experiments were designed to study the effect of hypophysectomy on experimental proteinuria in the rat. One hundred and nine adult males of the Slonaker-Addis strain were hypophysectomized by the para-pharyngeal approach. For treatment, materials were injected subcutaneously from day of operation to termination of experiment 24 days later. Dosages administered were: DOCA, 0.5 mg. daily; cortisone, 2.0 mg. daily; ACTH, 4.6 mg. (equivalent Armour standard-1-A) daily; and finally, growth hormone, 0.2 mg.; thyroxin, 0.005 mg.; and testosterone, 2.5 mg. daily. Proteinuria in the hour following the intraperitoneal injection of 4 dog units of hog renin in 4 cc. of saline solution was determined on the 10th, 17th, and 24th days postoperatively.

Hypophysectomy abolishes the proteinuria response to renin in the rat. Cortisone administration restores this proteinuria response to renin. DOCA and ACTH in the dosages used are largely ineffective. A combination of growth hormone, testosterone, and thyroxin maintains growth, but fails to restore renin proteinuria. In addition to renin proteinuria, the level of spontaneous proteinuria in the male rat is depressed by hypophysectomy, and none of the forms of replacement therapy results in a rise in this depressed level of protein excretion. Why DOCA and cortisone, equally effective in restoring renin proteinuria in the adrenalectomized rat, exhibit this marked difference in hypophysectomized rats is not clear. This phenomenon as well as the ineffectiveness of ACTH, and observations on organ weights, are discussed further.

LESIONS IN ACCESSORY SPLEENS. Béla Halpert and (by invitation) Wayne L. Eaton, Houston, Texas.

Abstract. Necropsy records of 600 patients, in whom special search for accessory spleens was made, revealed an over-all incidence of 10 per cent. There is general belief without substantial documentation that changes occurring in accessory spleens are identical with those occurring in the main spleen. It appeared of interest, therefore, to investigate the lesions occurring in accessory spleens. As a preliminary study the necropsy records with gross and microscopic preparations were surveyed in 31 male patients (25 white and 6 Negro) whose ages were between 32 and 75 years, and who had one or more accessory spleens. In 7 patients, no significant change was noted in either spleen. In both spleens of 14 patients with cardiac decompensation and of 3 patients with portal cirrhosis, chronic passive congestion was observed. In 1 patient each, the main and accessory spleens disclosed acute splenitis, periarteritis nodosa, leukemic change, lymphoblastoma (Hodgkin's type), or metastatic reserve

cell carcinoma. In both spleens of 2 patients, hemosiderin deposits occurred following massive blood transfusions. In every instance the lesion in the accessory spleen was similar to that in the main spleen.

CALCIFIED LESIONS IN HEART, SKELETAL MUSCLES, ARTERIES, AND LUNGS OF C3H MICE GIVEN PURIFIED LOW-PROTEIN DIETS. Benjamin Highman and (by invitation) Floyd S. Daft, Bethesda, Md.

Abstract. To study the effects of choline on the incidence of hepatomas (reported elsewhere), several series of C3H mice were given diet 1926, a purified low-protein diet deficient in choline with the following approximate percentage composition: sucrose, 86.05; casein, 4; Osborne and Mendel salt mixture, 4; Wesson oil, 3; cod-liver oil, 2; cystine, 0.5; anhydrous dextrose mixture, 0.25 (0.25 gm. contained 1 mg. of riboflavin); *p*-aminobenzoic acid, 0.1; and inositol, 0.1. To each 100 gm. of the above were added 3 mg. of calcium pantothenate, 1 mg. of niacin, and 0.5 mg. each of thiamine and pyridoxine. Weanling mice placed on this diet usually died in 4 to 8 weeks, but mice fed a purina dog chow stock diet for 2 to 5 months before beginning diet 1926 were more resistant. In addition to the anticipated cirrhosis, fatty changes, and ceroid deposition in the liver, the mice fed diet 1926 often developed yellowish white patches and streaks throughout the ventricular myocardium and silvery streaks in skeletal muscles, particularly the recti capitis. Microscopically, there were scattered focal lesions through the ventricular myocardium, rarely in the auricles, showing hyalinization, necrosis, and calcification of myocardial fibers with little or no inflammatory reaction and no associated vascular lesions. The lesions in skeletal muscles resembled somewhat those in vitamin E deficiency except for absence of an acute inflammatory reaction. Similar lesions occurred sometimes in the striated muscles of the esophagus. The lungs of many mice, after 2 months on the diet, showed extensive calcareous deposits in the interalveolar septa and along the surface of alveolar ducts and alveoli, but not in bronchi or bronchioles. Many arteries, particularly the large and medium-sized arteries in the kidneys, showed in the intima or media focal homogeneous basophilic deposits staining like calcium and provoking no apparent cellular reaction. Some mice showed, in addition, calcified lesions involving pulmonary veins, renal tubules, renal pelvis, perirenal fat, liver capsule, or other tissues. Similar necrotic and calcified lesions, usually less severe, occurred in albino Swiss mice fed diet 1926. The lesions were not prevented by choline or vitamin E supplements.

The lesions described differ in part, particularly in the variety of tissues affected, from those produced by other deficient diets. For example, the lesions in the arteries and muscles resembled those reported by Ashburn, Daft, Endicott, and Sebrell in rats given sulfaguanidine in purified diets. In the rats, however, comparable lesions were not found in the heart or lungs, the coronary arteries were chiefly affected, and the muscle lesions responded to vitamin E. In many respects the lesions in our experimental mice resemble metastatic calcification such as is found in hypervitaminosis D. While diet 1926 has no apparent excess vitamin D, the possibility must be considered that the metabolism of compounds like vitamin D may be disturbed in animals fed such abnormal diets.

THE EFFECT OF CORTISONE ON CERTAIN MAMMALIAN CELLS IN TISSUE CULTURE. Margaret Holden (by invitation), Beatrice Carrier Seegal, and Irene Ryby (by invitation), New York, N.Y.

Abstract. A tissue culture technic was used to study the effect of cortisone on splenic cells from the mouse, guinea-pig, and rabbit, and on cells from the buffy coats of rabbit bloods. The tissues came from normal animals of all three species, from guinea-pigs and mice infected with tubercle bacilli, and from guinea-pigs and rabbits

immunized with heat-killed tubercle bacilli. The method used was the double coverslip technic of Maximow. Cortisone acetate (Merck), cortisone vehicle, or tuberculin (PPD) were incorporated singly in the nutrient clot as follows:

Testing agent	μg. per tissue culture clot (2 drops)	Approximate dilution
Cortisone	0.025	1–4,000,000
Cortisone	0.0125	1–8,000,000
Cortisone vehicle		1–10
Cortisone vehicle		1–100
PPD	0.25	1–400,000
PPD	0.125	1–800,000

Eighteen explants were tested with every reagent at each experiment. The cultures were incubated at 37° C. for 1 to 4 days and the migration and growth of cells recorded at 24-hour intervals. Representative slides were fixed and stained for further study. The results indicate that 0.025 μg. of cortisone often inhibited the migration of leukocytes and the growth of fibroblasts derived from normal animals irrespective of species. Half the amount of cortisone gave little or no inhibition of these cells. Explants from animals infected with tubercle bacilli or immunized with this bacterium were usually inhibited by the concentrations of PPD tested. Explants of these tissues were also, quite regularly, moderately or markedly inhibited by 0.025 μg. of cortisone and were slightly inhibited in about one-half the experiments by 0.0125 μg. The cortisone vehicle was without effect on the explants.

EFFECTS OF RADIOACTIVE IODINE ON THE THYROID GLAND OF THE RAT. Simon Koletsky, Cleveland, Ohio.

Abstract. The uptake of I_{131} by the thyroid glands of rats on an iodine-free diet ranged from about 60 to 100 per cent of the injected dose. Maximum uptake occurred within 18 hours after injection. A single dose of 500 μc. resulted in virtually complete destruction of the thyroid gland, while 250 μc. produced severe damage and 100 μc. slight to moderately severe damage. The gland showed coagulation necrosis with obliteration of follicles and progressive fibrosis. The lesion was less marked in the poles and isthmus than in the body of the gland. Necrotizing vascular disease was observed, also injury to the parathyroid with the higher dosages. In rats studied up to 16 months after injection of the I_{131}, the ultimate appearance of the gland seemed to depend on the amount of initial damage. In more or less severe injury, compensatory hypertrophy and hyperplasia were usually inconspicuous. Although atypical cell groups were commonly observed, there were no acceptable instances of malignant transformation.

INTERSTITIAL CELL HYPERPLASIA IN THE ADRENOGENITAL SYNDROME. Benjamin H. Landing and (by invitation) Eli Gold, Boston, Mass.

Abstract. Patients with the adrenal hyperplasia form of the adrenogenital syndrome who live several years or more show a lesion previously reported as "bilateral adrenal cell tumor of the testis." Study of the testes of 3 such patients who died in infancy demonstrates that this lesion is actually extreme interstitial cell hyperplasia, and that this process differs from paraganglion cell or ectopic adrenal rest hyperplasia. The presumed endocrine mechanisms involved appear explicable on the basis of known facts concerning the hormonal control of the interstitial cells.

COMPLEMENT FIXATION IN ANIMAL NEOPLASIA. VI. DEVELOPMENT OF THE REACTION IN VARIOUS BREEDS OF RABBITS CARRYING THE BROWN-PEARCE CARCINOMA. Stuart W. Lippincott and (by invitation) Helen Thornton, Lester D. Ellerbrook, Mark C. Rhees, and Conrad T. O. Fong, Seattle, Wash., and Bethesda, Md.

Abstract. We have reported previously that positive complement fixation reactions were obtained upon the admixture of antigen (saline extracts of the Brown-Pearce carcinoma) and sera from New Zealand White rabbits carrying the neoplasm. Studies of this reaction have been extended to include tests with the sera of rabbits of several different breeds—Martin, Chinchilla, Flemish, Dutch, Havana, and Lilac. The sera of the majority of the animals developing tumors gave positive complement fixation reactions of approximately the same magnitude as those observed in the earlier studies. The positive reactions appeared at about 2 weeks after inoculation with the neoplastic tissue, and they increased until about 30 to 40 days after inoculation. The effects upon the reaction of such factors as serum-antigen ratio, serum concentration, and serum inactivation temperature were similar to those observed in studies with rabbits of the New Zealand White breed.

THE PECULIAR CHANGES OF NUCLEAR CHROMATIN IN VERRUCA VULGARIS. Herbert Z. Lund and (by invitation) Cecilie Leuchtenberger, Cleveland, Ohio.

Abstract. Hyden and Caspersson have attributed the increase of desoxyribose nucleic acid in the nuclei of verruca vulgaris to proliferation of virus. Evidence is presented to show that this is abnormal proliferation of nuclear chromatin (polyploidy).

BILIARY XANTHOMATOSIS (CONGENITAL ACHOLANGIC BILIARY CIRRHOSIS) DUE TO HYPOPLASIA OR PARTIAL AGENESIS OF BILE DUCTS. H. Edward MacMahon and (by invitation) S. J. Thannhauser, Boston, Mass.

Abstract. The term biliary xanthomatosis (xanthomatous biliary cirrhosis) was suggested to designate a clinical syndrome characterized by chronic jaundice, an enlarged liver, hypercholesteremia, and xanthomatosis. The syndrome is not common and most cases are seen in middle-aged females. Investigators today generally agree that the liver is the seat of the trouble, and that "pericholangiolitic biliary cirrhosis," which is characterized by a chronic inflammatory reaction about the terminal perilobular cholangioles and by a loss of the interlobular bile ducts, is the most common underlying lesion.

A male child, 10 years of age, appeared to show all of the clinical manifestations of this syndrome. From birth the patient had been jaundiced; there was intense pruritis; the liver and spleen were palpable; and the skin and the mucous membranes of the oral cavity were covered with xanthomata. The blood cholesterol was 1686 mg. per cent; phospholipids, 2313 mg. per cent; and the neutral fat, 2444 mg. per cent; each of these figures being from 10 to 20 times greater than normal. The serum, in spite of this very high neutral fat content, was transparent. An exploratory laparotomy (Dr. Swenson) revealed an enlarged liver and a gallbladder filled with dark green bile. Methylene blue injected into the gallbladder passed through the duct system into the duodenum. Cholangiograms (Dr. Saunders) showed an abnormally delicate extrahepatic biliary system in which the filling of the hepatic ducts emerging from the liver, in spite of their patency, was barely perceptible. A satisfactory biopsy specimen from the liver showed an absence of interlobular bile ducts. Liver cells were arranged in trabeculae, trabeculae were grouped into lobules, lobules were bordered in the usual way by portal areas, and lastly, junctional ducts were clearly discernible in the most peripheral zones of the lobules, but not a single interlobular bile duct was demonstrable. There was no obvious route for the elimination of bile from the lobules, and yet bile stasis was not conspicuous. Many portal areas were enlarged and showed a slight increase in fibrous tissue and mixed cellular infil-

tration. Strands of fibrous tissue passing from one portal area to another and from portal areas to central veins formed narrow partitions that divided the liver into segments of varying size. This summation of changes led to a minimal degree of what might be called "congenital acholangic biliary cirrhosis."

The association of biliary xanthomatosis with hypoplasia or partial agenesis of the bile duct system is an important observation. The pathologic histology of this anomaly and that of pericholangiolitic biliary cirrhosis have one important factor in common, namely, an absence of the interlobular bile duct system. This case not only draws attention to the importance of this part of the liver in cholesterol and phospholipid metabolism, but shows even more clearly the significance of a single defect in initiating so complex a syndrome, for here is an example of a liver showing normal growth and development in the absence of an interlobular bile duct system. Furthermore, all transitions could be seen between cells comprising the junctional ducts and those forming the trabeculae of the liver. These two observations would seem to indicate that liver cells and junctional ducts have a common origin, and that neither liver cells nor junctional ducts are derived from interlobular bile duct epithelium.

THE PRODUCTION OF CARDIOVASCULAR ANOMALIES BY ELECTROCOAGULATION IN CHICK EMBRYOS. Louis Manhoff, Jr. (by invitation) and Mychyle Johnson * (by invitation), San Antonio, Texas.

Abstract. A method has been developed by which selective cardiovascular anomalies of certain types, comparable to those occurring in man, can be produced in chick embryos. A high frequency current of diathermy type is used with a micro-electrode made from capillary glass tubing and platinum wire of 25 μ diameter. This electrode is manipulated by a simplified microdissector, assembled by mounting a mechanical stage on the rack of a compound microscope. The coagulation is performed under a stereoscopic dissecting microscope, coagulating a sharply restricted area.

Coagulation of the right sixth aortic arch was performed on 3-day-old chick embryos. Of 41 operated chicks, 26 survived beyond the 9th day and 16 of these lived to hatching. Of the 26 surviving chicks, 16 displayed a total of 21 anomalies, consisting of the following: stenosis of the right pulmonary artery, 12; absence of the right pulmonary artery, 2; absence of the right ductus arteriosus, 2; double aortic arch, 2; persistent truncus arteriosus, 1; interventricular septal defect, 1. A single anomaly, double aortic arch, occurred in 1 of 86 control chicks, and this was in a control group in which the actual operative procedure was performed except that the coagulating current was not turned on.

These experiments demonstrate that a focal lesion in the early embryo can result in malformations of the cardiovascular system. It is not inconceivable that similar focal lesions could be produced in embryos by certain environmental factors which are associated with experimental and human malformations. These experiments have also furnished data in support of certain embryologic principles concerned in the development of cardiovascular anomalies which have heretofore been based principally on indirect evidence. The method described is not difficult and could be applied to the study of a number of problems involved in the development of various congenital malformations.

MORPHOLOGIC, BIOCHEMICAL, AND IMMUNOLOGIC STUDIES ON SEVERAL STRAINS OF HISTOPLASMA CAPSULATUM. Angus McLaurin (by invitation), Parker R. Beamer, and Dorothy M. Tuttle (by invitation), Winston-Salem, N.C.

Abstract. The investigation comprised comparative studies of 17 strains of *H. capsulatum* isolated from fatal infections in infants, children, and adults from 5 states. Organisms were maintained on Sabouraud's agar at room temperature, 2 of the strains for about 7 years, and the most recently isolated for about 2 years. All

* Deceased.

cultures were transferred to fresh media every 2 months. Colonial and microscopic characteristics were studied with several media under variable atmospheric conditions and temperatures of incubation. None of the strains grew under anaerobic conditions, and the majority would not grow under increased tensions of carbon dioxide (5 to 10 per cent). All strains grew best at room temperature under aerobic conditions. Total mass of growth was less on blood agar than on Sabouraud's dextrose or corn meal agars. On blood agar all strains grew as flat, membrane-like colonies with numerous radiating sub-surface hyphae, and, except for 4 strains, no aerial hyphae. On Sabouraud's and corn meal agars all strains produced large, fluffy colonies with numerous aerial hyphae bearing characteristic tuberculate chlamydospores. Production of spores was earlier and in larger numbers on corn meal agar. Three different patterns were noted in giant colonies of the 17 strains on Sabouraud's and blood media, with the differences more distinctive on the former. Such differences were reproducible after animal passage. No significant differences in microscopic structures were found. All strains failed to produce acid from glucose, raffinose, rhamnose, mannose, fructose, galactose, lactose, maltose, sucrose, and inulin. Methyl red, Voges-Proskauer, indol, sulfide, gelatin-liquefaction, and citrate utilization tests were negative. In contrast, all strains hydrolyzed urea.

Three strains representing different growth patterns were selected for immunologic studies. After incubating in synthetic broth (Scheff), mycelial growths were dried *in vacuo*, macerated and suspended in saline solution, and formalinized (0.5 per cent final concentration). Antigens were diluted to match a no. 3 nephelometer tube, and rabbits were immunized by 9 intravenous doses. The sterile filtrates of these cultures were treated with alcohol and acetone to obtain corresponding fractionated antigens. Complement fixation tests were performed, using whole antigen and alcohol and acetone precipitated fractions from each of the 3 strains. Homologous and heterologous antisera fixed complement with complete antigen, as well as with both fractionated antigens from 2 of the strains. However, using alcohol and acetone precipitated antigens from the third strain, complement fixation occurred with heterologous antisera, but not with homologous serum. This paradoxical finding might be explained by postulating that a specific antigenic substance, common to all 3 strains, was not released into the medium during growth of the one strain, but that some soluble fraction which reacted only with heterologous serum was released. Such a substance could be: (1) a haptene corresponding to similar groupings on antigenic fractions of the other 2 strains; (2) it could be a second common antigen which, in the third strain, is shielded by other antigenic substances in such a manner that immunization with whole antigen fails to cause production of antibodies for that portion.

Mycelial growths from all strains were inoculated subcutaneously and intraperitoneally into mice, and animals were sacrificed 6 to 8 weeks later. Only the 3 most recently isolated strains produced lesions in the tissues, although the organisms were recovered from all animals. With the other 14 strains parasitization of tissue cells was noted without histologic lesions.

CLINICAL "CURE" OF DIABETES MELLITUS DURING INTERCAPILLARY GLOMERULOSCLEROSIS. J. F. A. McManus and (by invitation) H. G. Davis, Jr., and B. H. Bishop, Charlottesville, Va., Chicago, Ill., and Birmingham, Ala.

Abstract. The disappearance of hyperglycemia and glycosuria in a case of diabetes mellitus during renal failure, proved at necropsy to be due to intercapillary glomerulosclerosis, has been described and two similar cases have been mentioned. Subsequently, further cases have been collected from various centers, geographically separate, so that a total of 12 cases are available for study. These are described briefly in the present report. In one case there was pituitary necrosis with disappear-

ance of glycosuria and hyperglycemia 1 week ante mortem. In the other cases in which the pituitary body was examined the cellular structure was within normal limits. The adrenal glands were studied histologically in all cases. Satisfactory cortical tissue was always found. These studies dispose of functional hypophysectomy or adrenalectomy as an explanation of the amelioration of diabetes in the great majority of cases. Clinical "cure" of diabetes mellitus in intercapillary glomerulosclerosis may be considered as a variation in abnormal carbohydrate metabolism. It emphasizes the necessity of careful scrutiny of the history in every case of "non-diabetic" intercapillary glomerulosclerosis. In our experience, a history of diabetes mellitus has been found in every case of intercapillary glomerulosclerosis which was not diabetic at the time of death.

FATAL ATYPICAL ACID-FAST INFECTION. Ann Pollak (by invitation) and Victor B. Buhler (by invitation), Kansas City, Kans.

Abstract. We have recently observed 2 fatal cases of infection by an atypical acid-fast organism, with distinctive morphologic and bacteriologic features. Several other cases apparently belong to this group.

Case 1 (S. B.) This patient, a 21-year-old white male, became ill with a gradual onset of fever, weakness and increased fatiguability 14 months before death. Pancytopenia appeared and the temperature reached 103° F. The patient's course was steadily downhill despite the use of numerous antibiotics. The tuberculin test was negative. Necropsy revealed scattered large mesenteric nodes. The normal lymph node structure was replaced by partially calcified caseous and purulent masses. Similar caseous masses were seen in the spleen. Smaller miliary nodules were found in the lungs. Microscopic examination revealed poorly outlined lesions with some necrosis, large acid-fast bacilli, and but few giant cells.

Case 2 (D. L. C.). A 4-week-old infant was born 2 months prematurely, and did well at first on the usual premature regime. At 2 weeks of age, however, he developed cyanosis and dyspnea requiring continuous O_2 administration. His course from then on was downhill and after 10 days he was transferred to the University of Kansas Medical Center. He was admitted moribund with signs of pneumonia at both bases, and died 24 hours later. At necropsy the lungs showed diffuse bronchopneumonic consolidation. The other organs were grossly normal. Microscopically, the lungs presented inflammatory foci containing enormous numbers of large, elongated, acid-fast organisms. Similar lesions were found in a hilar node. Small miliary granulomas containing numerous acid-fast bacilli were found in the liver and in the malpighian bodies of the spleen. Numerous acid-fast organisms were also found free in the splenic pulp.

The organism recovered from the first case and observed in the second case is a large, beaded, strongly acid-fast bacillus that tends to occur in packets and clumps. Its appearance in tissues is very similar to that of *Mycobacterium tuberculosis*, although the individual organisms are much larger. It grows with relative ease on Petragnani's medium, but has a yellow colony. It produces a mild, self-limited disease in guinea-pigs. It is also mildly pathogenic for mice. Further studies on this organism are in progress. A similar organism has been recovered from several patients who are under observation.

WIDESPREAD FOCAL MYOCARDITIS INDUCED IN RABBITS BY MEANS OF PAPAIN SOLUTIONS INJECTED INTRAVENOUSLY. Theodore Robertson (by invitation), Aaron Kellner, and Alan Thal (by invitation), New York, N.Y.

Abstract. A striking focal myocarditis has developed in rabbits given papain in the form of a 5 per cent solution of the crude enzyme preparation in physiologic saline solution, passed through a Seitz filter, and injected intravenously in amounts

of 0.8 to 2.0 cc. per kg. The lesion was present in virtually every one of more than 40 animals injected, and in most instances it was widespread. No similar lesion was encountered in numerous control animals studied.

The earliest myocardial lesions were observed in rabbits killed 3 hours after the injection of papain and consisted of scattered focal areas where individual muscle fibers were swollen, granular, and eosinophilic. These degenerative changes progressed rapidly to complete necrosis within 24 hours. Polymorphonuclear leukocytes were present early in the areas of degeneration and necrosis, and, as the lesions evolved, considerable numbers of lymphocytes, large mononuclear cells resembling Anitschkow myocytes, and multinucleated giant cells were seen. Two days after injection, the necrotic muscle fibers began to disappear and an ingrowth of fibroblasts was evident. In animals that were permitted to survive longer than 4 days, the lesions consisted essentially of scattered focal areas where the myocardial fibers had been replaced by loose scar tissue containing an occasional giant cell. Careful search of both early and late lesions revealed no inclusion bodies. The lesions were distributed irregularly throughout the musculature of both auricles and ventricles and bore no obvious relation to blood vessels. It is noteworthy that in a few rabbits given large doses of papain there were, in addition to very extensive myocardial lesions, also a few small areas of degeneration and necrosis of skeletal muscle, particularly in active muscles such as the diaphragm and the masseters. No other visceral lesions attributable to papain were observed. A similar acute focal myocarditis and, to a lesser extent, myositis were produced in rats and mice given papain solutions intravenously. Crude trypsin solutions and crystalline trypsin in amounts of 4 to 10 mg. failed to produce comparable lesions when injected intravenously.

The papain-injected rabbits regularly developed a blood coagulation defect, which appeared to play no rôle in the pathogenesis of the myocarditis, since hemorrhages were not conspicuous in or about the myocardial lesions. Whether the myocarditis is due to the proteolytic enzyme present in the papain solution or to some other factor cannot be stated with certainty at the present time. In either case, the causative factor appears to have an unusual specificity for cardiac muscle, and perhaps also for other striated muscles.

THE VENOUS VALVE IN ENDOCARDITIS. Otto Saphir and Maurice Lev, Chicago, Ill.

Abstract. Because of the morphologic resemblance of the aortic valve and the valves of peripheral veins, the latter were studied microscopically in acute bacterial endocarditis and in instances of old (rheumatic) endocarditis. The structure of the normal valve was investigated and principally two layers, a "parietalis" and "lumenalis," were differentiated. Among 22 instances of acute bacterial endocarditis, changes in the valves of the femoral vein were encountered twice. In one a small number of polymorphonuclear leukocytes and some fibrin were found attached to the lumenalis. The lumenalis itself contained an edema-like material with very few inflammatory cells. Most of these were encountered in the base (commissural mound) of the valve. In the second instance, a portion of necrotic valve cusp was surrounded by masses of fibrin, polymorphonuclear leukocytes, and disintegrated red blood corpuscles. The differential diagnosis between thrombophlebitis in the region of the valve and venous endovalvulitis, most likely the result of bacterial embolization, is discussed. In 2 instances of old endocarditis, thickened cusps with bulb-like ends were encountered. In one of these a very recent red thrombus was found imposed upon the lumenalis.

OBSERVATIONS ON INTRA-SPLENIC OVARIAN TRANSPLANTS IN RABBITS. Edward C. H. Schmidt, Philadelphia, Pa.

Abstract. It has been demonstrated that various rodents are capable of inactivating the estrogens produced by ovaries transplanted into the spleens of castrate ani-

mals. In mice and rats these transplants grow into granulosa cell tumors or luteomas. Since the rabbit does not undergo the regular estrus cycles, as do mice and rats, it appeared interesting to see if like changes would occur in similar intra-splenic transplants. Ten virgin rabbits were castrated following pregnancy tests and approximately one-half of an ovary was transplanted to a pouch in the spleen. The animals were sacrificed at various intervals ranging from 25 to 372 days. In 3 animals sacrificed at 25, 32, and 36 days, there was marked reduction in the size of the fragments due to central atrophy. Numerous primordial follicles and evidence of proliferation were present in the peripheral portions of the remaining ovarian tissue. The grafts of 60 to 372 days' duration showed a gradual increase in size up to 2 cm. in greatest diameter. With increase in size an increasing luteinization occurred in the ovarian stroma, ranging from the clusters of rather large, pink-staining cells usually found in the ovarian stroma to a condition in which almost the entire transplant was composed of large lipoid-containing cells, which stained intensely with sudan III. An occasional vacuolated cell contained doubly refractive particles when viewed with polarized light. In the older grafts, numerous cysts measuring up to 5 mm. in diameter were present, most of which were lined by granulosa cells. Many of the larger cysts contained bloody débris. The uteri were small and the vaginas were usually ribbon-like. The endometria varied from being atrophic to showing evidence of estrogenic stimulation. The vaginas had flattened rugae but in the animals with grafts of long duration, the mucosa cells were high and active. It is believed from a study of the secondary sex organs that most, but not all, of the estrogens elaborated by the intrasplenic luteomas are inactivated by the liver.

STUDIES OF THE MECHANISMS OF CORTISONE INHIBITION OF GRANULATION TISSUE. David M. Spain and (by invitation) Norman Molomut, Valhalla and Brooklyn, N.Y.

Abstract. Previous studies have demonstrated that cortisone inhibits the formation of granulation tissue in wounds. In one of these studies cortisone was administered 48 hours after wounding and in these animals there were no significant differences in the quantity or quality of the granulation tissue as compared with non-treated controls. It therefore seemed likely that one or more of the mechanisms influenced by cortisone in the inhibition of wound healing might be present in the first 48 hours. There are indications that cortisone significantly decreases the level of circulating fibrinogen.

With this in mind, 20 Swiss albino mice (20 to 25 gm.), housed in individual cages and kept under controlled conditions, were wounded on their backs so that each mouse had two similar wounds 0.5 cm. in diameter. These mice were injected subcutaneously with 2.5 mg. of cortisone daily (injections beginning 1 day previous to wounding). At the time of wounding drops of free flowing blood from the tails of non-treated mice were placed on the left wound of each mouse. After this had clotted, both wounds were covered with collodion. Wounds were observed daily and on the fifth day all animals were sacrificed. Wounds were excised completely, sectioned, and stained with hematoxylin and eosin.

Daily gross observation revealed contraction of the wounds on the left side whereas the right-sided wounds did not contract at all. Microscopic examination revealed growth of granulation tissue on the surface of the wound immediately beneath the blood clot. There was insignificant granulation tissue growing in from the sides of the wounds on the left side. The surfaces of the right-sided wounds contained no granulation tissue while the edges appeared similar to the left-sided wounds. It would appear, therefore, that cortisone interferes with something necessary for the formation of granulation tissue that is brought to the wound by the circulating blood. One of these substances may be fibrinogen. Studies are now being carried out in which fibrinogen is being added to the wounds of cortisone-treated mice.

MORPHOLOGIC CHANGES IN INTACT AND IN HYPOPHYSECTOMIZED RATS INJECTED WITH CORTISONE. Robert B. Stebbins (by invitation) and Herbert C. Stoerk, Rahway, N.J.

Abstract. Since it appeared possible that some of the effects of cortisone are due to an action antagonistic to the growth hormone, it seemed of interest to see whether some of the changes produced by cortisone in the presence of the pituitary gland would fail to appear after hypophysectomy. Comparison was made between groups of intact and of hypophysectomized male rats, half of which were injected subcutaneously with 3 mg. of cortisone daily for 4 weeks. This treatment caused cessation of growth in the intact animals, while it produced a weight loss of about 20 per cent of the initial body weight in hypophysectomized rats. Pathologic changes in intact rats injected with cortisone were marked atrophy of the adrenal glands, extreme loss of lymphoid tissue, increased glycogen content of liver cells, increased weight of fat tissue, loss of granulation and swelling of renal tubular epithelial cells, and a striking reduction in width of the epiphyseal cartilage. Adrenal atrophy concerned exclusively the inner zone. There was virtually complete loss of both sudanophilic and birefringent materials from the atrophic cells. Cells of the inner cortex showed hydropic change and in many instances contained lipochrome pigment. Loss of lymphoid cells concerned lympho-epithelial and lymphoid tissue alike. Renal tubular changes, resembling those in desoxycorticosterone-treated rats, were frequently but not regularly found. In the adrenal glands of uninjected, hypophysectomized rats, loss of lipoid from the inner cortex was marked but not complete. In this group, loss of coarsely granular birefringent particles from the glomerular zone was also evident but was not paralleled by loss of sudanophilic material. In the inner cortex of cortisone-treated, hypophysectomized rats, there was nearly complete disappearance of lipoid from the cells. In contradiction, the glomerulosa contained abundant, finely granular, birefringent particles and sudanophilic material. This zone was strikingly wider and more conspicuous in the hypophysectomized cortisone-injected rats than in the uninjected controls. Heart, kidney, liver, and pancreas appeared larger in hypophysectomized, cortisone-treated rats than in the untreated controls. It appears, therefore, that cortisone prevents the development of the microsplanchnia characteristic of pituitary insufficiency. With the possible exception of its action on the adrenal cortex and on cartilage, cortisone produces essentially the same changes in hypophysectomized as in intact rats.

THE POLYSACCHARIDE NATURE OF CORPORA AMYLACEA. Howard D. Steele, Gordon J. Kinley, and Cecilie Leuchtenberger (all by invitation), Cleveland, Ohio.

Abstract. Cytochemical studies of corpora amylacea of the prostate, lung, nervous system, and pineal gland showed that they contain a polysaccharide with 1,2 glycol grouping. When tested for the presence of desoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA), they were found to be negative. The lack of DNA and RNA suggests that the corpora amylacea might be other than cellular in origin.

MALIGNANT MIXED MÜLLERIAN TUMOR (MIXED MESODERMAL TUMOR OF THE UTERUS): A STUDY OF ELEVEN CASES. William H. Sternberg and (by invitation) Wallace H. Clark, Jr., and Robert C. Smith, New Orleans, La.

Abstract. From January, 1946, to January, 1951, 11 malignant mixed tumors of the uterus were seen in post-pubertal women in the Charity Hospital of Louisiana at New Orleans. These neoplasms, which we regard as histogenetically and clinically similar, have been described under several names, such as mixed mesodermal tumor, sarcoma botryoides, and carcinosarcoma. All tumors contained two or more types of sarcoma, and most of them contained carcinomatous elements also. The sarcomatous elements included endometrial sarcoma, leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma, chondrosarcoma, and unclassified sarcoma. The carcinomatous elements

were endometrial adenocarcinoma, papillary cystadenocarcinoma, tubal adenocarcinoma, epidermoid carcinoma, and unclassified carcinoma.

The presence in such tumors of mesodermal elements not normally found in the uterus has suggested to various authors several theories of histogenesis. These include mesoderm brought down by the wolffian duct in its descent, inclusion of displaced cells of the primitive myotomes, müllerian inclusions of multipotential cells, and neoplastic metaplasia of the endometrial stroma. Our study does not support any of the "cell rest" theories of origin, but suggests that the tumor may arise from mature tissue of müllerian origin, most frequently the endometrial stroma. The evidence favoring this view is as follows: 1. Histologically, normal endometrial stroma closely resembles embryonic müllerian tissue (and embryonic mesenchyme in general) and is probably the least differentiated of müllerian structures. 2. Bonser and Robson, applying 20-methylcholanthrene to the endometria of mice, produced malignant tumors which, as judged from their illustrations, are very similar to some of ours. 3. Ten of 11 neoplasms in this investigation contained regions resembling typical endometrial sarcoma, and grossly appeared to originate within the endometrium. 4. Tissues such as striated muscle and cartilage, not present in the normal uterus, were often found surrounded by, and apparently originating from, nests of endometrial sarcoma. Adjacent to one tumor, striated muscle appeared to be arising from non-neoplastic endometrial stroma. 5. The varied epithelial structures present in these tumors were all compatible with müllerian origin, and often appeared to be differentiating within nests of endometrial sarcoma.

The patients' ages ranged from 42 to 67 except for one 13-year-old girl. The presenting sign in all was abnormal vaginal bleeding and more than one-half of the patients had friable masses at the cervical os. Ten of the 11 patients died, or were known to have metastatic tumor, within 1½ years after diagnosis. The clinical manifestations and pathologic findings of the mixed müllerian neoplasms were remarkably uniform and the prognosis was consistently poor. The sarcomatous components of these tumors covered the broad spectrum of elements seen in other mixed mesenchymal tumors. However, the epithelial constituents were sharply limited to those varieties of epithelium normally derived from müllerian tissue, in this respect differing from true teratomas.

PATTERNS OF SUDAN-STAINABLE FAT IN HUMAN OVARIES OF DIFFERENT AGE GROUPS WITH SPECIAL REFERENCE TO STROMAL HYPERPLASIA. S. Tannhauser, Buffalo, N.Y.

Abstract. The conception of stromal hyperplasia of the ovarian cortex as a possible source of hyperestrinism has lately assumed increased importance in its relation to thecoma as well as in its bearing upon endometrial carcinoma. Several authors have tried to link endometrial carcinoma with a higher incidence of stromal hyperplasia; however, the main difficulty encountered in this attempt is to differentiate sharply between the normally occurring proliferative changes of the ovarian cortex in the menopausal age group and true hyperplasia. Since, in the follicular structures as well as in the thecomas and granulosa cell tumors, hormonal activity is found linked with sudan-stainable fat, the distribution of such fat in human ovaries of different age groups was studied, in the hope of finding a truer indication of the presence of hormones.

Two hundred human ovaries ranging from patients 18 to 84 years of age were studied with hematoxylin and eosin stains, connective tissue stains, and sudan IV stains. In adult life up to 35 years of age, sudan-stainable fat centered around the follicular structures and their derivatives, corpora lutea and albicantia. Stromal hyperplasia was rare; when it occurred no interstitial or inter-cellular fat was present. In the age group between 35 and 45 years, stromal hyperplasia developed. Fat was present mainly in the follicular structures but was beginning to appear in scattered

cells or minute nodular foci in the cortex. Past 45 years, fat disappeared from the scantier follicular structures with increasing age but appeared in smaller and larger nodules or in diffuse plaques in the cortex in practically all ovaries of this group. All transitions from minute foci, comprising a few cells, to true thecomas were demonstrated. The most marked nodular, diffusely interstitial, and perivascular fat accumulations were found at the age of 50 to 60 years, but sometimes fat was present up to 80 years of age (in a case of carcinoma). In non-carcinomatous cases fat became scanty around 70 years of age. While the 7 cases of endometrial carcinoma studied in this group showed very marked nodular and diffuse fat accumulations in the ovarian cortex, noticeably more than in the non-carcinomatous cases, their number so far is too small to allow definite conclusions as to the interdependence of these findings.

Sudan-stainable fat is present in ovaries of the young age group in and around the follicular structures; in the premenopausal and menopausal age it slowly switches to the ovarian cortex, appearing in and around the cells of the cortical stroma. It is believed that these fat studies present a truer picture of hormonal activity in the ovarian cortex than the concept of stromal hyperplasia alone.

ADRENAL CYSTS. H. R. Wahl, Kansas City, Kans.

Abstract. Since the literature would indicate that cysts of the adrenal glands are rare, the occurrence of 9 in a series of 13,996 necropsies justifies a brief study and report. These cysts were usually unilateral (only one being bilateral) and affected the left adrenal gland more often (4 of 6). They were all found in adults. They were all accidental findings and were twice as frequent in males. The cause and nature of the cysts are often obscure, because of the frequent desquamation and disintegration of the lining cells. They occur in three groups. The largest group were cysts derived from lymphatics, either as single large lymph cysts or multiple lymphangiectases or hygromas, 2 belonging to the former and 2 to the latter. The second most frequent type was the degenerating form resulting from disintegrating adenomata or local hemorrhage and necrosis. There were 3 cysts arising apparently from adenomata. This form is to be distinguished from the so-called cystic adenoma in which the adenomatous character is easily obvious. The smallest group in this series were 2 cases of multilocular, calcifying, cystic adenomas, one of which was bilateral. This cystic nature was striking while the glandular nature was only revealed on careful study. That 9 cases should be found in such a small group of necropsies should impose some doubt on the unusual rarity of these lesions as suggested by the scanty reports in the literature.

ACUTE DIFFUSE GLOMERULONEPHRITIS IN MAN AND IN EXPERIMENTAL ANIMALS: A COMPARISON. Douglas Waugh (by invitation) and Robert H. More, Edmonton and Montreal, Que.

Abstract. Acute diffuse glomerulonephritis in man and that induced experimentally in animals have common morphologic, clinical, and immunologic features which point to certain pathogenetic factors. The kidneys of humans dying shortly after the onset of acute glomerulonephritis show histologic changes similar to those seen in rabbits killed 8 to 10 days after development of clinical nephritis induced by two injections of bovine serum gamma globulin. This experimental nephritis is morphologically similar to that described in rabbits and rats injected with a variety of foreign proteins, including "nephrotoxic" sera. The glomerular lesions are typified by epithelial and endothelial swelling and proliferation, basement membrane alteration, reduction in capillary bed, and, often, by crescent formation. Human acute glomerulonephritis manifests hematuria, proteinuria, and cylindruria, beginning about 10 days after an upper respiratory infection, usually streptococcal. Experimental nephritis of the type outlined above presents similar urinary abnormalities 6 to 9

days after the first injection of foreign serum. This coincides with the development of hypersensitivity, as manifest by the appearance of a positive Arthus reaction and specific precipitins in the serum, together with an elevation of serum globulin. In both human and experimental nephritis there is elevation of blood urea nitrogen as obstructive glomerular lesions develop. The rise in serum globulin in experimental nephritis is due to elevation of both gamma and beta fractions, mainly the former. As in human glomerulonephritis, this often results in reversal of the albumin-globulin ratio.

Hypersensitivity is the factor common to all experimental glomerulonephritides, but this state may be induced by a variety of antigens. No single type of antigen is specifically related to the production of the lesions. Consequently it appears that other factors that modify the state of hypersensitivity are of importance in pathogenesis. For example, exposure of animals to cold enhances the lesions and is associated with increased food intake, while dietary restriction appears to inhibit them. These observations find their human counterpart in the outbreak of acute nephritis in Amsterdam in 1945 during a period of dietary rehabilitation.

Experimental globulin nephritis in rabbits does not appear to be mediated by anti-kidney antibodies. The importance of such antibodies in any of the experimental nephritides cannot be regarded as firmly established, since experiments in which they have been employed have involved the injection of foreign protein. Furthermore, the occurrence of urinary and morphologic manifestations of renal damage in both nephrotoxic and foreign serum nephritis 6 to 9 days after serum injection argues for an actively developing hypersensitivity rather than passive transfer. Moreover, in both types of nephritis the antigen antibody mechanism of the recipient animals must be intact. It is suggested, therefore, that the pathogenesis of both nephrotoxic and foreign serum experimental nephritis is dependent on active hypersensitivity. The manifold similarities between the human and experimental diseases indicate that hypersensitivity may be of importance also in the pathogenesis of glomerulonephritis in man.

THE CONTRASTING COLOR REACTIONS OF MALIGNANT TUMORS. Emil Weiss, Chicago, Ill.

Abstract. The colors of some dyes can be reduced in contact with tissues. Malignant tumors reduce more rapidly than normal tissues or benign tumors. Small particles of tissue and vigorous shaking hasten considerably the process of reduction. Some of the reducible dyes are only slightly affected by normal tissues or benign tumors, while malignant tumors cause a very rapidly progressing decolorization or highly contrasting changes of the original color. The dyes, with the exception of litmus, are used in dilutions 1:10,000, dissolved in 35 per cent isopropyl-alcohol. Benzo blue, chlorazol black E, naphthogene blue 2B, sodium 2,6-dichlorobenzenone-indophenol, and sodium 2,6-dichlorobenzenoneindo-3′-chlorophenol are completely decolorized by malignant tumors, while normal tissues and benign tumors retain the original color, only slightly lighter. Cotton blue, litmus, and Poirrier's blue are changed by malignant tumors to a light rose from the original blue. Trypan blue is changed by malignant tumors to a pale blue from the original violet. Tissues in amounts of 0.25 to 0.5 cc., cut up in small particles, suffice. The tissues are placed in tubes, covered with 5 cc. of the dye, and corked. They are shaken vigorously for 1 to 2 minutes and then the color changes are noted. Tubes containing malignant tumors have a turbid supernatant fluid while normal tissues and benign tumors have a clear supernatant fluid. A dye control for the respective dye, a normal control with normal tissue or benign tumor, a malignant control with a mixture of malignant tumors serve for comparison of the results. Tissues containing a small number of malignant tumor cells or of low degree of malignancy may give a less distinct reaction, or the color changes may develop more slowly. Malignant tumors of any type give

turbidity and contrasting color reactions. The contrasting color reactions may be considered as one of the characteristics of malignant tumors.

STUDIES ON METASTASIS. INCREASE IN NUMBER OF METASTASES WITH TIME DUE TO SUCCESSIVE RELEASES OF EMBOLI FROM A PRIMARY TUMOR. Irving Zeidman, Philadelphia, Pa.

Abstract. As recently reported, the number of metastases increases the longer a primary cancer is present in the host. Is this increase with time the result of successive releases of embolic cells, or is it due to the escape of tumor emboli of different sizes in a single shower? To test the hypothesis that emboli escape at different times, experiments were planned using transplants of fibrosarcoma 241 in C57 black mice. This tumor regularly produces spontaneous pulmonary metastasis. Subcutaneous implants were removed at intervals, and the mice were sacrificed several weeks later. Of mice that had borne primary tumors for 11 to 12 days, about 50 per cent were found to have pulmonary metastases; therefore, 11 to 12 days was assumed to be the time of initial release of emboli. In subsequent experiments, the implants were removed 11 to 12 days after inoculation in one series of mice, and after 16 days in another series. All mice were sacrificed 32 days after inoculation. Significantly more metastases were found in those mice that had borne the tumor for the longer time. It is therefore concluded that the increasing number of metastases with time is dependent upon successive releases of emboli rather than upon a single shower of emboli from the primary tumor.